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## THE BIOLOGY OF *PODISUS SERIEVENTRIS* UHLER, IN CAPE BRETON, NOVA SCOTIA<sup>1</sup>

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### Abstract

The biology of *Podisus serieventris* Uhler, and its role in an outbreak of the black-headed budworm, *Peronea variana* Fernald, in Cape Breton, N.S., are described from studies carried on in 1930 and 1931. There is but one complete generation of *Podisus* a year, and adults of both sexes hibernate. The eggs are laid in late June, July and early August, the incubation period ranging between 10 and 15 days. There are five nymphal stages, requiring about 45 days, on the average, for the attainment of the adult condition. In these respects particularly, the life history of *Podisus serieventris* in Cape Breton differs from its life history in Massachusetts, where four nymphal stages and three annual generations and the hibernation of females only, have been reported.

The species conforms satisfactorily to Dyar's Law, the average growth ratio of individuals studied in 1931 being about 1.28. The first-stage nymphs feed on unhatched eggs of their own species, and upon the juices of coniferous and deciduous foliage, but were not induced to feed upon small caterpillars. Nymphs were able to complete the first instar on a purely vegetable diet, but died before the second moult when the same diet was continued. Older nymphs, fed for some time on animal food, were not able to attain the adult condition when supplied with plant food alone. This indicates the dependence of the species upon animal food; the food consumption of the various stages is briefly summarized. Evidence is presented which suggests the utilization, by *Podisus*, of a toxic secretion in overcoming their prey.

The rather limited value of *Podisus* as a control factor in the outbreak of *Peronea variana* in 1930 and 1931 is described. The decline of the *Peronea* population in 1931 caused a corresponding mortality in the *Podisus* population, by starvation.

### Introduction

This paper includes the more important biological data which, together with a study of the external anatomy of the adult, were incorporated in a thesis "The biology of *Podisus serieventris* Uhler, with especial reference to its predatory habits," submitted to the Faculty of Graduate Studies and Research, McGill University, in April 1932. The paper deals with the species in Cape Breton Island, N.S., in relation to the black-headed budworm, *Peronea variana* Fernald.

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Contribution from the Dominion Entomological Laboratory, Fredericton, New Brunswick, Canada. This paper was constructed from a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, Montreal, Canada, in April 1932, in part fulfilment of the requirements for the degree of Master of Science.

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### *Distribution and Habitat*

The predacious pentatomid, *Podisus serieiventris* Uhler, is distributed throughout the maritime provinces, Quebec and Ontario, through the New England states to New Jersey and Pennsylvania in the east, also in Illinois and Minnesota, and in Colorado, Wyoming and Montana in the west. It has also been recorded from Vancouver Island. The species is particularly an inhabitant of shade and forest trees. Brief references to the usefulness of *Podisus serieiventris*, as an enemy of tree defoliators, occur in the literature; Kirkland (7, 8) studied the species in Massachusetts, where it preys upon the larvae of the gipsy moth.

Severe damage in the balsam-spruce forests of southern Cape Breton, caused by *Peronea variana*, was reported to the Dominion Entomological Branch in July, 1929. The outbreak was investigated in 1930, by Mr. R. E. Balch, entomologist-in-charge, Dominion Entomological Laboratory, Fredericton, N.B., assisted by the writer. Among the factors of control operating against the budworm, *Podisus serieiventris* gave promise of some importance. Particular attention was centered on *Podisus* in 1931, to elucidate the interrelations between host and predator.

### *Methods of Study*

Field observations and collections of bugs were made during the course of population studies of *Peronea*. Early in the season, when the insects were very small, trees were felled so that the crown rested on a large cotton mat, 16 ft. square. The limbs were cut off, and carefully examined, and insects which dropped out of the foliage onto the mat were also collected. Later it was found advisable to delimb the standing trees; climbing irons were employed in ascending the trees, and the limbs were lowered down onto the mat by means of stout cord. In this manner, the budworm and stink-bug populations were carefully followed from June till the middle of September. Detailed information on the biology of *Podisus* was obtained by rearing individuals in shell vials from time of hatching until after maturity. A 7 by 9-ft. silk tent, set up at Grand River, Cape Breton, served as a field laboratory.

### *Identification*

Individuals collected at St. Peters, C.B. in 1930, were identified as *Podisus serieiventris* Uhler, by Mr. G. S. Walley, Division of Systematic Entomology, Ottawa. Additional specimens, collected at Grand River in 1931, were given the same determination by Dr. H. M. Parshley, Smith College, Massachusetts. The question of authoritative determination is important, for at least three writers have referred to the confusion between *Podisus serieiventris* Uhler and *P. maculiventris* (Say.). Van Duzee (20) writes: "Mr. Kirkland reports this (*P. serieiventris*) as 'by far the most common representative of the genus' in Massachusetts, but his description seems to refer to the form given here as *maculiventris*." It should be noted, however, that Kirkland had his material identified by Professor Uhler, the author of *serieiventris*. Morrill (11) states. "..... whether or not the form known to some as *serieiventris* be ultimately

considered as a species distinct from *P. maculiventris*, its habits appear to be the same as those of the latter." Again, Parshley (see Britton, 3) says: "The form described here (as *serieventris*) agrees with Uhler's description and type, but it is not the *serieventris* of some authors. The black spot of the corium appears to be a constant feature and together with the rather blunt pronotal angles and short ventral spine will serve to distinguish it from the other members of the genus." The quotation of Van Duzee is especially relevant, as Kirkland has published, so far as the writer is aware, the only extensive study of *P. serieventris* in America. One inclines to the view that Kirkland's specimens were probably identified correctly. If so, the details in the biology of the species in Cape Breton differ greatly from those recorded for Massachusetts, as described later.

Baker (2) distinguishes between the two species as follows:

*P. maculiventris*: "Basal spine of abdomen long, extending between hind coxae; lateral angles of pronotum prominent and acute; length 10-12.5 mm.; color greyish-brown."

*P. serieventris*: "Basal spine of abdomen short, not extending between hind coxae; lateral angles of pronotum blunter."

"Second antennal segment one-third longer than the third; lateral angles of pronotum not prominent; color greyish-brown, corium with a dark spot; length 8-10 mm."

## Description

### Adult

Professor Uhler's original description (19) was transcribed by Kirkland (7). The specimens taken in Cape Breton agree very well with Uhler's description. The majority of individuals are blackish-gray in color; a few are grayish-brown, with the embolium and distal portion of the corium of a reddish hue. In a few individuals the cephalo-lateral margins of the pronotum are reddish, but in the majority of specimens the margins are yellow. The female bugs are considerably larger than the males. Measurements of a number of adults of both sexes, selected at random from the collection, are shown in Table I.

TABLE I  
BODY MEASUREMENTS OF *Podisus serieventris*

Dimension measured	Male adults			Female adults		
	Number measured	Range, mm.	Average, mm.	Number measured	Range, mm.	Average, mm.
Head width	12	1.20- 1.54	1.42	17	1.42- 1.64	1.55
Humeral width	11	4.6 - 5.5	5.0	10	4.9 - 6.3	5.7
Length to tip of abdomen	11	8.5 - 9.9	9.0	10	9.1 -11.4	10.6
Length to apex of hind wing at rest	11	9.5 -11.1	10.3	10	10.2 -12.7	11.7

*Egg (Fig. 1)*

The eggs are metallic bronze in color, caldron-shaped, tapering slightly toward the posterior pole. In height they range from 1.20 to 1.25 mm., in diameter from 0.90 to 0.96 mm. The circular lid or operculum, with its dense armature of short, stiff, dark spines, covers the anterior pole of the egg. Numerous curved rod-like outgrowths, the chorionic processes, the "seminal cups," or "elongated cup-shaped micropyles" of Leuckart, surround the operculum. They vary in number from 10 to 14, with an average of 12 for 55 eggs examined. Earlier investigators believed that the spermatozoa entered the egg through these chorionic processes. Heidemann (6) states that more recent opinion inclines to the view that they serve to ventilate the interior of the egg. The walls of the egg are ornamented with shorter and more sparsely placed spines. The surface of the egg is nearly bare near the posterior pole.

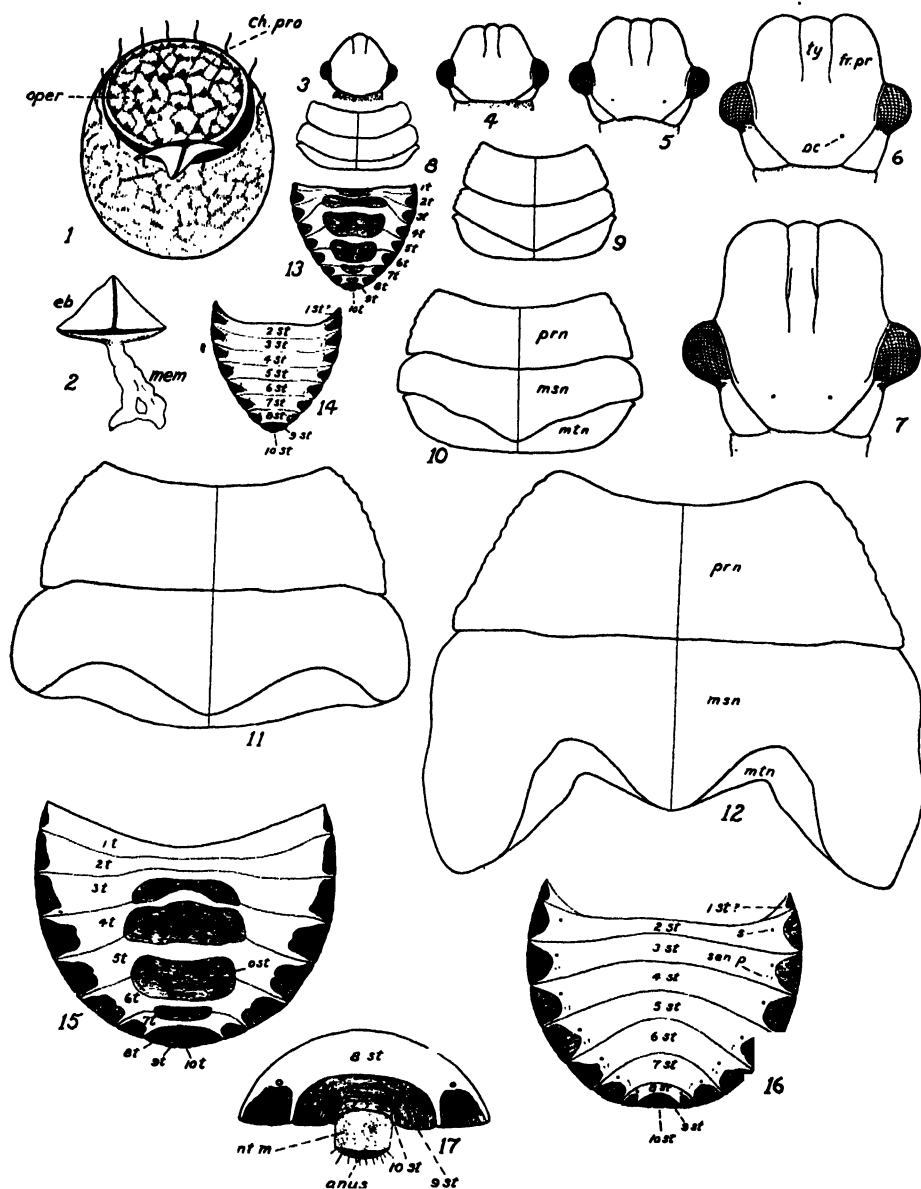
The eggs of *P. serieventris* can be readily distinguished from those of *P. maculiventris* and *P. modestus*, species of the same genus found in Cape Breton. The eggs of *maculiventris* are slightly smaller, and darker, and have a denser armature of longer spines than those of *serieventris*, and the chorionic processes are about one-third longer. The eggs of *modestus* equal in size those of *serieventris*, but are distinguished by their lighter color and smooth surface, and by the greater curvature of the chorionic processes.

*The Nymphal Stages*

Kirkland (7, 8) reared *P. serieventris* from the egg stage to the adult, in Massachusetts, in 1896 and 1897, and found but four nymphal stages, which he describes in detail. The writer found five nymphal stages in Cape Breton, which are described below.

*First instar.* Length 1.6 to 1.7 mm., width 1.05 to 1.1 mm. Form ovate, tapering more rapidly toward caudal end; convex above and below. Head dark brown, rounded on occipital margin (Fig. 3); eyes dark red, not prominent; anterior margin of tylus rounded, extending beyond frontal processes; ocelli not apparent, even in head boiled in potash. Head impunctate, sparsely clothed with microscopic yellow hairs; short, stout hairs on margin anterior to eyes; head disposed dorso-ventrally. Antennae 1.05 mm. long; four-segmented; brown, whitish at joints; segments in order of length, 1, 3, 2, 4, the latter being the longest; segment 1 stout, curved basally; segments 2 and 3 thickened distally; segment 4 widest near middle; ring joints not distinct; all segments pubescent. Beak four-segmented, stout, reaching to posterior coxae; segment 3 shortest, 1 and 2 subequal, 4 longest; apex membranous, bearing minute hairs. Labrum extends to distal margin segment 1.

Thorax (Fig. 8) dark brown dorsally, with median white sulcus; pro- and meso-nota subequal; metanotum reduced medially, widening laterally; short hairs borne on lateral margin of thorax; impunctate, but transversely furrowed. First spiracle on posterior margin proepimeron, second on posterior margin mesoepimeron. Legs brown; coxae widely separated; femora



FIGS. 1-16. 1. Hatched egg of *Podisus serieventris* Uhler. 2. Egg-burster, removed from the hatched egg. 3-7. Dorsal view of head capsule:—3, first instar; 4, second instar; 5, third instar; 6, fourth instar; 7, fifth instar. 8-12. Dorsal view of thorax:—8, first instar; 9, second instar; 10, third instar; 11, fourth instar; 12, fifth instar. 13. Dorsal view of abdomen of first instar. 14. Ventral view of abdomen of first instar. 15. Dorsal view of abdomen of fifth instar. 16. Ventral view of abdomen of fifth instar. 17. Caudal segments of abdomen, fifth instar, enlarged, ventral view.

ABBREVIATIONS:—ch. pro., chorionic process; eb, egg-burster; fr. pr., frontal process; int. m., intersegmental membrane; mem, membrane at base of egg-burster; msn, mesonotum; mtn, metanotum; oper, operculum; oc, ocellus; ost, ostiole; prn, pronotum; s, spiracle; sen. p., sensory puncture; st, sternum; t, tergum; ty, tylus (clypeus).

widened distally, swollen near apex, anterior tibiae widest at middle, middle and hind tibiae uniform in width; on inner surface of anterior tibiae, near apex, and on anterior margin at apex, are short spurs, of consolidated setae; tarsi two-segmented, second about twice as long as first; tarsal claws light brown, curved, paired; pulvilli scoop-shaped; empodium consists of a spatulate structure bearing two long apical hairs. Sternum weakly sclerotized.

Ground color of abdomen (Fig. 13) pale yellow; on dorsum are seven brown, sclerotized medial areas; first and second narrow; third constricted medially, widening laterally; fourth to seventh roughly rectangular, the posterior two smaller, and having a medial sulcus. The paired, oval-shaped openings or ostioles of the scent glands are situated between the fourth and fifth, and the fifth and sixth, abdominal terga. Connecting the hind margins of each pair of openings is the transverse conjunctiva, which, according to Moody (10), "is a fine line marking the scar through which the gland lining is pulled out when a molt takes place." Between the third and fourth terga are two narrow slits, but these are apparently not connected with the scent glands. Along the lateral margin of dorsum is a series of brown sclerotized areas; there is one area for each segment up to the eighth; the first area is small, triangular, adjacent to the metanotum; the second to the eighth are semi-elliptical, decreasing in size posteriorly; the ninth and tenth segments are completely sclerotized. Mesad of the lateral areas are 10 to 12 bands of red, the anterior ones directed cephalo-mesally, the posterior ones caudo-mesally. On the sternum of the abdomen are lateral sclerotized areas corresponding in number, size and color to those on the dorsum; mesad of these is a row of short, irregular, transverse red bands.

There are presumably eight abdominal spiracles (Fig. 14). The first is very minute in the last three stages, and can be detected only after staining (acid fuchsin), which causes the tracheal trunk to show up as a very fine tube; it was not observed in the first three instars, but presumably it is located on the dorsum of the first segment, mesad of the lateral triangular area; the remaining seven spiracles are located on the sterna of the succeeding segments, mesad of the lateral sclerotized areas. Five pairs of sensory punctures are present, as in all other stages, behind the spiracles on the third to the seventh segments, inclusive.

*Second instar.* Length 2.3 to 2.9 mm., width 1.7 mm. Form elliptical, convex as in 1. Head (Fig. 4) blackish brown, more quadrate than in 1, occipital margin nearly straight; eyes red, prominent, head narrowed in front of eyes; tylus extending slightly beyond frontal processes, constricted posteriorly; frontal processes marginated laterally and anteriorly, a few microscopic hairs being borne on the margin. Tylus and frontal processes coarsely punctate, the punctures extending onto frons and vertex, but finer on these latter regions; coarse punctures bounding the mesal margin of the eye; occiput impunctate. Ocelli not present. Antennae 1.5 mm. long, segments 2 and 4 subequal; ring joints apparent at bases of segments 3 and 4; segments 2 and 3 pubescent, segment 4 with longer hairs. Basal segment

of beak black, others brown; segment 1 subcylindrical, 2 enlarged apically, 3 and 4 decreasing apically; beak flattened dorsally, convex ventrally. Labrum extends to basal portion second labial segment. In this and following instars, the head is held in more or less of a horizontal position.

Metanotum narrowed medially and laterally (Fig. 9); lateral margin of pronotum serrate, that of mesonotum slightly serrate. Thorax uniformly, coarsely punctate, somewhat rugose medially. Thoracic pleura more heavily sclerotized than in 1; sterna slightly sclerotized. Coxae closer together than in 1; tibiae broadly grooved outer surface; otherwise the legs are as in 1.

The abdomen has undergone some modification; the first medial dorsal area partly obliterated, only its lateral extremities remain; the sixth and seventh areas less distinctly divided medially. There is a more conspicuous lateral margin. The transverse red bands are longer, extending to the medial areas. On the mid-ventral region of segments 4 to 8, inclusive, are sclerotized brown areas, the first two very small; the third largest, and the last two successively smaller. The sclerotized areas on the dorsum of the abdomen are coarsely punctate; those on the sternum are impunctate.

*Third instar.* Length 3.8 to 4.6 mm., width 2.5 to 3.1 mm. Elliptical, widest at third or fourth abdominal segment. Head more quadrate than in II; ocelli not evident on intact head, but two minute transparent areas are visible on the moulted head capsule of this instar (Fig. 5). Antennae 2.3 mm. long; segments in increasing order of length, 1, 3, 4, 2.

Pronotum expanded laterally; posterior margin of mesonotum extends back in V-shaped lobe, the rudiment of scutellum; on either side of medial lobe is a slight projection, the rudiment of the wing pad; metanotum partially overlapped by mesonotum (Fig. 10). Thorax rugose medially, coarsely punctate laterally and posteriorly; on each segment, on either side of the medial line, is a small smooth area. Color black, or black with two brown spots on each side of the pronotum, and a smaller spot on each side of mesonotum. Coxae near mid-ventral line; legs as in II.

- Abdomen much as in II; the first medial area on the dorsum is represented only by faint lateral portions; the second is very small or obliterated, the others as in II. The red bands are more extensive, some extending completely across the dorsum.

*Fourth instar.* Length 5.8 to 6.7 mm., width 3.4 to 4.1 mm. Ovate, widest at third abdominal segment. Head (Fig. 6) nearly rectangular, longer than broad; ocelli distinct; antennae 3.1 to 3.4 mm. long, antennal segments as in III; beak as in III.

Caudolateral lobes of mesonotum enlarged, extending to caudal margin of metanotum; slight rounded lobes on caudolateral margins of metanotum (Fig. 11). Color of thorax variable; may be dark brown or black, with large brown areas on pro- and mesonota, and occasionally small brown spots on metanotum; or, more rarely, light brown may predominate, with dark brown



or black on mid-dorsal line and margins. First tarsal segment relatively longer than in preceding instars.

Lateral triangular area on first abdominal segment partially concealed by lobes of mesonotum; first and second medial areas almost obliterated; transverse red bands more extensive than in III.

*Fifth instar.* Length 7.5 to 9.0 mm., width 4 to 5 mm. Form as in IV. Tylus partially divided, posteriorly, into three parts (Fig. 7); ocelli distinct, eyes prominent. Antennae 4.3 mm. long, segment 3 relatively longer than in IV. Segments 1, 3 and 4 of beak subequal; segment 2 longest.

Lobes of mesonotum extend back onto third abdominal segment, overlying all but the mesal portions of the metanotal lobes (Fig. 12). Legs as in IV; swellings on femora less distinct.

First three lateral areas on abdomen reduced to narrow strips along the margin (Fig. 15). The eighth, ninth and tenth segments are completely sclerotized, dorsally; the ninth and tenth segments are completely sclerotized ventrally.

The coloration is variable; commonly, head and thorax are black with brown markings, antennae blackish-brown; coxae, trochanters and base of femora pale brown, remainder of legs dark brown. Ground color of abdomen cream, transverse bands red, lateral and medial areas are dark brown to black. The abdominal glands of the nymph atrophy during the fifth stage, and the metathoracic glands of the adult begin to develop at the same time; the two sets of glands, while performing the same function, are not morphologically related in any way (10).

### Head Capsule Measurements

That the occurrence of five nymphal stages in Cape Breton is quite normal, is evidenced by the following data on head capsule measurements. Taylor (17) quotes Dyar (1890) as follows: "If two sets of observations show a different number of stages for the same insect but each follows its own progression, we may conclude that this variation is actual; but if either set shows a lack of regular progression that one we must regard with suspicion." The measurements shown in Table II were made on the exuviae of bugs which were reared individually after the first moult, so the instar corresponding to each cast skin was definitely known. The width of the head, between the narrow annular sclerites along the mesodorsal margin of the eye (Figs. 3-7), was found most convenient for measurement.

In calculating the theoretical head widths, the average ratio of increase was employed. The growth ratio between the first and second stages works out to 1.257, between the second and third stages to 1.323, between the third and fourth to 1.296, between the fourth and fifth to 1.230. The average of the four ratios is 1.277. The theoretical widths of the later stages were calculated by multiplying the average width of the first stage by successive powers of the ratio 1.277.

TABLE II

FREQUENCY TABLE SHOWING THE MEASURED HEAD WIDTHS OF THE NYMPHAL STAGES OF *Podisus serieventris* UHLER, IN MM.

	1st instar		2nd instar		3rd instar		4th instar		5th instar	
	Width	No.	Width	No.	Width	No.	Width	No.	Width	No.
	.53	1	.60	1	.82	1	1.07	1	1.30	1
	.54	5	.64	2	.84	1	1.09	1	1.35	1
	.55	3	.65	1	.85	1	1.13	1	1.38	1
	.56	6	.66	5	.86	1	1.16	4	1.41	2
	.57	11	.67	1	.88	4	1.19	2	1.42	1
	.58	6	.69	1	.90	1	1.20	5	1.46	1
	.59	2	.70	1	.91	2	1.21	2	1.49	3
	.60	1	.71	5	.92	2	1.22	1	1.51	6
			.73	4	.93	3	1.24	3	1.53	1
			.74	6	.94	1	1.25	1	1.54	3
			.75	2	.95	3	1.26	3	1.56	1
			.76	2	.96	2	1.27	3	1.57	2
			.77	2	.97	1	1.29	3	1.58	1
			.78	1	.98	1	1.32	1	1.59	1
					.99	3			1.62	1
					1.00	1				
					1.02	1				
					1.03	2				
					1.06	1				
Total no.		35		34		32		31		26
Range	.53-.60		.60-.78		.82-1.06		1.07-1.32		1.30-1.62	
Arith. mean	.564		.709		.938		1.216		1.496	
Calc. mean (R = 1.277)	.564		.720		.918		1.172		1.496	
Prob. error of mean	± .00194		± .00512		± .00697		± .00715		± .01005	
Standard deviation	.0170		.0443		.0585		.0591		.076	
Coeff. variation	3.01		6.25		6.23		4.86		5.07	

TABLE III

HEAD WIDTHS OF SUCCESSIVE INSTARS OF *P. serieventris*, IN MM.

Sex of bugs	2nd instar	3rd instar	4th instar	5th instar
Males				
No. 1	.73	.88	1.16	1.46
No. 2	.60	.82	1.09	1.38
No. 3	.71	.95	1.22	1.54
No. 4	.65	.90	1.16	1.51
No. 5	.71	.93	1.21	1.49
No. 6	.73	.88	1.07	1.30
No. 7	.74	.93	1.19	1.41
Range	.60-.74	.82-.95	1.07-1.22	1.30-1.54
Average	.696	.899	1.157	1.441
Females				
No. 1	.74	.95	1.20	1.54
No. 2	.66	.86	1.13	1.49
No. 3	.67	.85	1.16	1.42
No. 4	.73	.96	1.26	1.51
No. 5	.70	.94	1.19	1.35
No. 6	.73	.93	1.24	1.51
Range	.66-.74	.85-.96	1.13-1.26	1.35-1.54
Average	.705	.915	1.197	1.470

There is some overlapping between the instars, as shown by the ranges. Considering, however, the actual means of the five instars in relation to the calculated means, it is apparent that there is a close approximation to a uniform growth ratio throughout the developmental stages. The standard deviation for each of the five instars is relatively small compared to the corresponding mean, as shown by the low coefficients of variation. The low probable error of the mean (computed by the Gaussian formula), gives evidence of a very fair degree of precision in the value of the means.

The successive head widths of 13 individuals were obtained, after the first moult. They are shown in Table III, the individuals being separated according to sex, which was determined at the final moult.

The number of individuals whose measurements are given in Table III is not as extensive as the number included in Table II, and the ranges for the instars are smaller. Because of the smaller number, the averages are less reliable than those given in Table II, although the differences are small. It will be noted that the female nymphs, on the average, are slightly larger than the male nymphs. The female adults are somewhat larger than the males, as shown previously.

### Seasonal History

#### *Emergence from Hibernation*

Kirkland (7) found that all males in his rearing cages died in the autumn, without entering the soil for hibernation. He concluded that the females, fertilized in the fall, are the only sex to hibernate. Morrill (12) writes: "Pentatomids are among the earliest insects to emerge from hibernation in the spring, although apparently only a small percentage passes the hibernating period successfully. Both sexes hibernate in many, if not all, species."

The time at which *P. sericeiventris* emerges from hibernation, in Cape Breton, was not determined. In 1931, the first balsam fir tree was examined June 1, and a male adult was found in the foliage. Altogether only 12 overwintering adults were found, 4 males and 8 females, although balsam trees were felled onto the cotton mat, and their foliage examined, every few days. The last of the overwintering adults was collected July 29, and none survived after August 5.

Newly emerged adults sucked the juices from the new growth (the needles and the stem) of balsam fir, and the juices of *Peronea variaria* larvae, in rearing vials. Although copulation was not observed in the case of overwintering adults, placed together after their capture in the field, the writer believes that copulation normally takes place during June and July. What was apparently an exceptional case of copulation occurred August 23, both the male and the female being newly matured bugs. They were observed *in coitu* at 1.30 p.m., at 4.30 p.m., at 9.30 p.m. and at 8.00 a.m. August 24, but had separated at 8.45 a.m. It is possible that the pair remained *in coitu* from 1.30 p.m. August 23 to 8.00 a.m. August 24, a period of over 18 hr.

### Oviposition

Oviposition presumably follows soon after copulation. Eggs were found in the field early in July, though some must have been present late in June because first stage nymphs were found July 2. The eggs are deposited in masses, each egg resting at a slight inclination, and are cemented to each other and to the surface upon which they are deposited. They were most frequently found on the new growth of balsam fir. It seems probable that the adults are attracted by the succulent foliage, which serves also as a source of nourishment for the first stage nymphs. Eggs occurred less frequently on the old foliage of balsam, and occasionally on alder foliage. Four egg masses were found on spruce foliage, in the Gaspé balsam-spruce forest, in 1932; in this region, the most abundant insect available as food is the white spruce sawfly, *Diprion polytomum* Hartig, feeding on the spruces. It seems probable that the eggs tend to be laid near the source of food, which may be purely accidental in that the adults may lay their eggs while in search of food.

The number of eggs laid by the females was not determined exactly. Only two females laid eggs in captivity, one laying 23 in one mass, the other laying 48 in four lots of 14, 14, 14 and 6 respectively, the first lot August 28, the second September 3 or 4, the third September 8, and the final lot September 21. This female was the one observed in copulation soon after maturity, mentioned above as an exceptional case; her period of oviposition was also exceptional. Kirkland states the females lay 50 to 60 eggs.

The number of eggs to a mass ranged from 4 to 23, with an average number of 11 for 27 masses examined.

### Incubation

The incubation period of the species in Massachusetts, in 1896 and 1897, was 8 to 10 days, according to Kirkland. In Cape Breton the period was somewhat longer (Table IV), and of five egg masses under observation the period ranged from 10 to 15 days.

TABLE IV  
INCUBATION PERIOD OF EGGS OF *P. serieventris*

Eggs in masses	Date deposited	Date of hatching	Period, (days)	Average daily mean temperature, °F.
23	July 28	Aug. 7	10	66.6
14	Aug. 28	Sept. 11	14	61.8
14	{ Sept. 3 p.m. or Sept. 4 a.m. }	Sept. 18	{ 14 or 14½ }	60.1
14	Sept. 8	Sept. 23	15	—
6	Sept. 21	Oct. 4	13	Room temperature

The average daily mean temperatures during the periods were calculated from the daily maxima and minima, recorded in the tent laboratory.

### *Process of Hatching*

The fully developed embryo lies within the egg shell with its head directed at right angles to the long axis of the body, and immediately under the operculum. The beak, antennae and the legs are folded upon the venter. Inside the egg shell is a peculiar T-shaped structure known as the anchor process or egg-burster which consists of a transverse bar and a median spur. A tough, transparent membrane lies at the base of the transverse bar, and between the apex of the median spur and the extremities of the transverse bar (Fig. 2). Attached to the base of the anchor process is a thinner membrane, which, according to Heidemann (6) "envelopes the young larva before it emerges from the egg." Before hatching, the anchor process partly covers the dorsum of the thorax, the median spur fitting over the mid-dorsal sulcus, and the transverse bar resting approximately along the line of union between the head and pronotum. The transverse bar thus underlies an arc of the periphery of the operculum.

When eclosion begins, the operculum is loosened all around its periphery, except for a small arc adjacent to the anterior margin of the clypeus of the embryo. This small attachment serves as a hinge on which the operculum swings upward. The operculum is apparently loosened by the upward pressure of the anchor process; Muir (see Butler, 4) considers that the egg-burster actually cuts through the chorion, around the edge of the operculum. If this is so, the transverse bar would presumably perform the cutting of the egg shell. As the nymph emerges, the operculum swings upward, and the egg-burster is drawn forward along the mid-dorsal line of the thorax and head, and then backward along the mid-ventral line. The peristaltic movements accompanying eclosion are almost imperceptible. As soon as exposed, the legs and antennae are exercised moderately. The usual sequence is as follows: the anterior legs are first exercised, then the antennae (often aided by the anterior legs) and finally the middle and posterior legs. After a short interval the legs are extended to the upper surface of the operculum, and the nymph draws itself from the egg shell. The integument of the newly emerged nymph is very soft and pale, and in the head region a continual throbbing may be observed. Usually the nymph rests on the operculum until its integument hardens and darkens.

The operculum falls back into place and the egg-burster usually protrudes through the aperture. The membrane at the base of the egg-burster is much shrunken after hatching; it is not definitely connected to the egg shell, but frequently adheres to its inner surface.

Five nymphs were timed, in the process of eclosion. The first loosening of the operculum was regarded as the starting point, and eclosion was considered complete when the nymph rested on the operculum. The observations are shown in Table V.

The percentage of hatch, based on the laboratory records and examination of field-collected egg masses, was over 90%. An unusual condition existed,

TABLE V  
TIME OF ECLOSION IN *P. serieventris* UHLER

Individual	1	2	3	4	5
Time clapsing before legs and antennae were released, min.	5	6	7	7	8
Time clapsing up to resting on operculum, min.	10	9	11	12	13

in that the first nymphs to emerge from an egg mass frequently sucked remaining unhatched eggs. This is described more fully below.

### Duration of the Stages

Nymphs were reared gregariously after emergence, until the first moult. In most instances, all the eggs of the same egg mass hatched within a few hours at the most; frequently, several nymphs would be emerging at the same instant. To a lesser degree, this uniformity of development among individuals from the same egg mass was also true of the successive moults.

After the first moult, most nymphs were placed in separate vials, and records on feeding and development were taken until their maturity. The individuals from two egg masses were reared collectively throughout the developmental stages, one lot in a lantern-globe cage, and the other lot in a large shell vial.

Data on the duration of the first stage are shown in Table VI. The first stadium ranged from 5 to 10 days. During the time that first-stage nymphs normally occurred in the field (July and the first half of August), the first stadium ranged from 5 to 8 days, with an average of approximately 6 days.

Data on the duration of the later stages, obtained from the individual rearings, are shown in Table VII. It will be observed that male and female bugs had practically the same duration, in the several stages.

TABLE VI  
DURATION OF THE FIRST STAGE OF *P. serieventris* UHLER

Number of individuals	Date of hatching	Date of first moult	Days spent in first stage	Average daily mean temperature, °F.
13	July 8	July 13	5	72.7
5	July 9	July 14	5	72.6
6	July 13 (a.m.)	July 19 (p.m.)	6½	66.3
12	Aug. 7	Aug. 14	7	65.2
1	Aug. 7	Aug. 15	8	65.7
7	Sept. 11 *	Sept. 20	9	59.2 **
2	Sept. 11 (a.m.) *	Sept. 20 (p.m.)	9½	59.2 **
9	Sept. 18 *	Sept. 28	10	Room temperature
9	Sept. 23 *	Oct. 2	9	Room temperature

\*The irregularity of eggs occurring at this time of the season has been noted under the heading "oviposition."

\*\*Temperature records, as well as other field records, were ended September 18, thereafter the nymphs were exposed to room temperature.

TABLE VII

DURATION OF THE LATER STAGES OF *P. serieventris* UHLER

Number of individual	Sex of bug*	Second stadium	Third stadium	Fourth stadium	Fifth stadium	Date bug matured
1	Male		6	8	12	Aug. 4
2	Female		6	10	13	Aug. 8
3	Male		8	6	18	Aug. 9
4	Female		8	7	14	Aug. 10
5	Female		8	8	12	Aug. 13
6	Male	7	6	8	12	Aug. 15
7	Male	8	6	9	11	Aug. 16
8	Male	8	6	8	13	Aug. 18
9	Female	11	6	8	11	Aug. 18
10	Female	8	6	10	13	Aug. 19
11	Female	7	8	8	14	Aug. 19
12	Female	7½	5	9	15	Aug. 20
13	Female	8½	6	9	18	Aug. 25
14	Male	6	8	8	14	Aug. 26
15	Female	9	6	8	25	Sept. 1
16	Female	7	9	10	16	Sept. 1
17 **	Female	7	8	8½	17	Sept. 23
18 **	Male	6	9	10	19	Sept. 27
19 **	?	8	10	9½	19½	Sept. 30
20 **	Male	10	9½	11½	18½	Oct. 2
Male bugs { Range		6-10	6-9½	6-11½	11-19	
Average		7½	7½	8½	14½	
Female bugs { Range		7-11	5-9	7-10	11-25	
Average		8	7	8½	15½	

\*The sex of individuals was determined after the final moult.

\*\*Individuals Nos. 17 to 20, inclusive, were fed on animal food alone; all others were provided both animal and vegetable food.

The data obtained from the two collective rearings are summarized in Table IX. It will be observed that the earlier moults were very close in point of time, among individuals from the same egg mass. As development continued, the cumulative effect of small differences among individuals became more pronounced; in lot B, there was a spread of 11 days between the earliest and latest moult into adult stage.

The total developmental period, from hatching until the final moult, was determined for 23 individuals. The data are shown in Table X, and relate to those nymphs (whose more detailed histories are included in Tables VII and IX) which were under observation throughout the whole developmental period. The developmental period ranged from 37 to 55 days, when hatching occurred in early July, and from 47 to 58 days, when hatching occurred in early August. The average period of the 23 individuals was 45 days. Very nearly the same average period is obtained by totaling the average duration of the five stadia, as shown in Table VIII.

TABLE VIII

AVERAGE DURATION OF THE FIVE STADIA IN *P. serieventris* UHLER

	1	2*	3*	4*	5*	Total
Stadium	6	7½	7½	8½	14½	44
Male, days	6	8	7	8½	15½	45 (approx.)
Female, days						

\*Table VII.

TABLE IX

SUMMARY OF LIFE HISTORY OF TWO LOTS OF NYMPHS REARED COLLECTIVELY

Lot	1st moult		2nd moult		3rd moult		4th moult		5th moult	
	Date	No.	Date	No.	Date	No.	Date	No.	Date	No.
A. . . . . 6 eggs										
Hatched July 12	July 19	6	July 24	6	July 30	5*	Aug. 7	4	Aug. 19	1
							Aug. 8	1	Aug. 20	4
B. . . . . 5 eggs	Aug. 14	4	Aug. 21	4	Aug. 29	2	Sept. 7	1	Sept. 23	1
Hatched Aug. 7	Aug. 15	1	Aug. 23	1	Aug. 30	2	Sept. 8	2	Sept. 24	1
					Sept. 7	1	Sept. 10	1	Sept. 26	1
							Sept. 19	1	Sept. 29	1
									Oct. 4	1

\*One of the third-stage nymphs escaped July 28.

TABLE X

TOTAL DEVELOPMENTAL PERIOD, *P. serieventris* UHLER, IN DAYS

Date of hatching	Date of final moult	Total developmental period	Sex of bug	Date of hatching	Date of final moult	Total developmental period	Sex of bug
July 8	Aug. 15	38	Male	July 13	Aug. 20	38	Female
July 8	Aug. 16	39	Male	July 13	Aug. 20	38	Female
July 8	Aug. 18	41	Female	Aug. 7	Sept. 23	47	Female
July 8	Aug. 19	42	Female	Aug. 7	Sept. 23	47	Female*
July 8	Aug. 19	42	Female	Aug. 7	Sept. 24	48	Female
July 8	Sept. 1	55	Female	Aug. 7	Sept. 26	50	Female
July 9	Aug. 18	40	Male	Aug. 7	Sept. 27	51	Male*
July 9	Aug. 20	42	Female	Aug. 7	Sept. 29	53	Female
July 9	Aug. 25	47	Female	Aug. 7	Sept. 30	54	? *
July 13	Aug. 19	37	Male	Aug. 7	Oct. 2	56	Male*
July 13	Aug. 20	38	Male	Aug. 7	Oct. 4	58	Female
July 13	Aug. 20	38	Female				

Range: 37-58 days      Average, 45 days

\*These individuals were fed on an animal diet alone, during the last four stages. All others were provided with both animal and vegetable food.

### Process of Moulting

Feeding generally ceases two or more days before moulting occurs. This cessation is necessary, because the chitinous parts of the salivary and pharyngeal pumps are cast out and replaced at ecdysis. Before ecdysis, the white areas turn dull gray, the integument is tightly stretched, and motions are jerky and uncertain.



The nymph prepares for moulting by spreading its legs widely, thrusting its head forward, the labium beneath the thorax, and the antennae beneath the thorax or to the sides. The head capsule splits along the paracephalic sutures, and the corneas of the compound eyes separate from the head capsule on the dorsal, anterior and anteroventral margins. The thorax splits along the mid-dorsal sulcus, and the pronotum is drawn away from the mesonotum. In the moulting of the fifth instar, the wing pads are elevated and the mid-dorsal split extends to the apex of the scutellum.

When ecdysis begins, the lateral halves of the thoracic terga are forced apart, exposing the thorax of the succeeding instar. The thorax is considerably humped, and the shortening of the body, accomplished by this humping, enables the head to be withdrawn from the old capsule. The thoracic segments are released by a series of peristaltic movements. The labium and antennae are drawn from their old coverings by the same movements that effect the release of the thorax and abdomen. The mandibular and maxillary setae project beyond the tip of the labium, both pairs curving lateroventrally, the former more strongly than the latter. As the setae are withdrawn a white sheath is slid from each, a phenomenon which is apparently the same as that which Snodgrass (15) found during moulting of the cicada. The basal segments of the legs are soon exposed, and the soft integument near the femorotibial joint alternately collapses and resumes the normal contour, a process which apparently assists in the release of the tarsal segments. The legs are then put through a series of moderate exercises, thus gradually gaining strength. The old chitinous linings of the tracheae are pulled through the spiracles of the new instar; they are readily observed being drawn through the thoracic spiracles, appearing as long, tapering white tubes. The abdomen is withdrawn from the collapsing skin by a series of bending movements, and frequently the hind tibiae are employed to thrust back the exuviae.

When the fifth instar moults, the delicate wings are exposed. The scutellum, horizontal at first, soon curves up apically, possibly to ensure more rapid hardening of the wings. The hind wings and the membranous portion of the fore wings are white, wrinkled and sac-like, becoming lamellate in a few minutes; this change may be assisted by a patting and stroking action of the posterior tibiae. The scutellum soon drops back into position. The genital segment of male adults protrudes at first, but is soon telescoped within the preceding segments. Two fifth stage nymphs, moulting into the adult, required 14 and 23 min. respectively, from the first splitting of the thoracic terga to the complete shedding of the exuviae.

#### *Number of Generations*

It has been pointed out that overwintering adults may be found in the trees during June, July and the first few days in August. Adults of the current generation appear quite early in August. In 1930, the first adults occurred in the field laboratory August 6; in 1931 the first adult in the laboratory rearings appeared August 4, and the first field-collected adult

of the current generation was taken August 14. Newly matured adults are recognized by their fresh appearance, and the entirety of the wing membranes; the wing membranes of overwintering adults are usually quite tattered. Most individuals of the *Podisus* population were in the adult stage by the end of August, though a few retarded individuals matured during September, and even in October (indoors).

Early maturing adults did not generally copulate in the late summer. Presumably this function takes place after the emergence of the two sexes in the spring. The one female that was known to be fertilized shortly after her maturity, in August, laid her eggs in August and September. Due to the lack of insect food, and to the low temperatures, there is little likelihood that the ensuing nymphs, which emerged during September and October in glass vials, would have been able to survive under field conditions. It seems improbable that there was more than one complete generation, in Cape Breton, in 1930 and 1931.

Observations in the Berry Mountain Brook district of central Gaspé indicate a similar life history of *P. serieventris* in that region, to that in Cape Breton. Overwintering adults, including one male, were collected early in July and again in the third week of August, 1932. Field-collected eggs hatched July 12 and September 9. Development was slow, as indicated by observations on a few individuals in vials, kindly supplied by Mr. W. A. Reeks. The duration of the first stage was 11 days; of the second, 15 days; of the third, 17 days; and of the fourth, 11 days. The individuals died before the fifth moult. Fifth-stage nymphs appeared early in September in the field; one of these moulted into the adult stage early in October, in the laboratory. The eggs which hatched September 9 were apparently deposited by one of the longer-lived overwintering females, as there was insufficient time for a generation to have matured and deposited eggs by the first of September. In all probability there was but one complete generation in central Gaspé, in 1932.

-In Massachusetts, Kirkland reared two generations of *P. serieventris* between the last of June and the last of September; he believed that the progeny of the overwintering adults had matured by the last of June, and concluded that there were three generations a year in that region.

### *Hibernation*

The time at which the adults enter into hibernation was not determined. Adults were seen in the field, in October 1930, at St. Peters. Field work at Grand River in 1931 ceased September 18, and at that time adults were still in the trees. In the absence of direct observation the writer presumes that the adults burrow into the forest litter in Cape Breton, as stated by Kirkland to be the case in Massachusetts. Both sexes hibernate in Cape Breton.

### *Sex Ratio*

The sex ratio, based on laboratory rearings and collections in the field in September 1931, before hibernation commenced, was between .55 and .65. The number of adults secured was not extensive enough to justify a more exact statement of the ratio.

### **Value as a Predator**

#### *Habits of First Stage Nymphs*

The first stage nymphs are definitely gregarious. For the first day or so after emerging, they remain huddled on the egg mass, those on the outside with their heads directed inwards. Nymphs in rearing vials ranged out over the foliage a day or two after emerging, and sucked the juices from the new foliage of balsam fir and white spruce, and from the leaves of alder and lilac. Almost any part of the leaf was punctured; in a few cases, the setae were applied to the stomata of balsam needles, but this may have been without significance. Newly emerged nymphs were occasionally found sucking from unhatched eggs of the egg mass from which they originated. Morrill (12) states that first-stage nymphs of *Pentatoma ligata* Say have the same habit, and examination by the writer of several egg masses of *Podisus modestus* indicated a similar habit for this species. The operculum of sucked eggs is not loosened, and the shell almost invariably collapses after the contents is withdrawn. Of 203 *P. serieiventris* eggs collected at Grand River in 1931, 19 had been sucked; of 48 eggs collected on white spruce in Gaspé in 1932, 5 had been sucked. About half of the collapsed eggs were practically empty, one-quarter contained a dried yellow substance, and the remainder contained dried irregular bodies in which a suggestion of segmentation was detected. Apparently most of the collapsed eggs had undergone little or no development at the time they were sucked, and judging by the simultaneity which characterizes hatching in the same egg mass, it seems probable that these eggs were infertile. There had been some development in about one-quarter of the collapsed eggs, evidenced by the dried segmental contents, and in these instances the young nymphs may actually have killed the embryos. It is of course possible that the embryos had previously died, and the young nymphs merely withdrew the unused food contents of the eggs.

There has been considerable discussion relative to the feeding habits of the first-stage nymphs of species of *Podisus*. Whitmarsh (23) writes of *P. maculiventris* as follows: "These insects are entirely predaceous, except possibly to a slight extent during the first instar when they may suck plant juices if they feed at all, which thus far is unproved." Stoner (16) describes the habits of first-stage nymphs of the same species, in the presence of celery leaf-tyer caterpillars; the nymphs did not feed on the caterpillars for 15 days after hatching, though some appeared to feed on celery sprigs, and showed cannibalistic tendencies as well. Kirkland (7) states that the first stage nymphs of *P. serieiventris* were fairly active five days after hatching, without once having had food. He also found that the nymphs fed on eggs of the

gipsy moth, but died if given no other food, and concluded that their death was due to insufficient nourishment.

Concerning several species of pentatomids studied in relation to the Mexican bean beetle, Plummer and Landis (14) write as follows: "First-stage nymphs of all species of pentatomids observed failed to feed on larvae of *E. corrupta*. As soon as the first molt occurred the nymphs attacked the larvae."

The writer placed small *Peronea variana* caterpillars in rearing vials with first-stage nymphs of *Podisus serieventris*, but in no instance did the latter feed upon the caterpillars. Experiments were carried out to determine (1) whether nymphs could pass the first stage without feeding at all; (2) whether they could subsist on vegetable food alone; (3) what stage of their development they could attain when the vegetable diet was continued.

(1) Thirteen nymphs were placed in vials without food. They were examined daily, and dead nymphs removed to prevent others from feeding upon their juices. All nymphs were feeble after the third to the fifth day. Two individuals lived for 4 days, 1 nymph for 5 days, 4 nymphs for 6 days, 2 nymphs for 7 days and 4 nymphs for 9 days. None, however, were able to survive till the first moult.

(2) In the ordinary rearing experiments, the majority of the nymphs survived the first stage when foliage was supplied. As a special check on the rate of survival, 40 newly emerged nymphs were placed in vials in lots of 14, 13, 9 and 4 respectively. Balsam, white spruce, alder, lilac or cherry foliage was supplied, and replenished every two or three days. Dead nymphs were removed as soon as found. Nine nymphs died in the first stage, 6 to 10 days after hatching occurred. Thirty-one nymphs (about 78%) moulted the first time, 7 to 10 days after hatching; the average duration of the first stage was 9 days.

(3) The 31 survivors, then in the second stage, were supplied with foliage only. Not one lived to moult the second time, but all died from 1 to 15 days after the first moult; the average length of life in the second stage was about 6 days. The total failure of these nymphs to survive until the second moult cannot be attributed to unsatisfactory conditions in the vials, because in the rearing experiments where animal food was provided as well as vegetable food, the normal mortality at this time of life was not over 10%. The death of these individuals was apparently due to the inadequacy of vegetable food, during the second stage.

#### *Feeding of Later Nymphal Stages*

Kirkland (7) found that at the various stages *P. serieventris* consumed larvae at the following rates, on the average: first instar, 4 larvae; second instar, 6 larvae; third instar, 4 larvae; fourth instar, 11 larvae; adult, 5 larvae. In the 30 days between hatching and hibernation, 30 larvae were killed, an average of one a day.

. Preliminary feeding experiments, in 1930, indicated that the bugs killed one *Peronea variana* larva every three days, as an average rate. More

extensive records were obtained in 1931, and the following insects were used as host forms: *Peronea varians* (all larval stages), *Ellopiia fuscicollis*, *Hyphantria cunea*, *Galerucella alni*, and a spittle bug (*Clastoptera* sp.) from alder. Twenty nymphs were reared individually, after the first moult, on both animal and vegetable food; 12 of these nymphs finally moulted into the adult form. Four nymphs were reared individually to the adult stage on animal food alone, and two lots of bugs were reared collectively from the egg to the adult stage on an animal and vegetable diet.

Without giving the detailed records, Table XI summarizes the more important observations on the food consumption of the last four nymphal stages, based on the individual rearings where animal and vegetable food was provided.

TABLE XI  
SUMMARY OF DATA ON FOOD CONSUMPTION OF LAST FOUR NYMPHAL STAGES OF  
*P. sericeiventris* UHLER

	2nd instar	3rd instar	4th instar	5th instar
Maximum number of larvae consumed by one individual	3	4	8	11
Minimum number of larvae consumed by one individual	1	1	2	3
Average number consumed	2½	3¼	4½	6½

The greatest number of larvae consumed by one nymph during the last four instars was 19; the least number was 12; and the average number was 15. This is equivalent to a little more than one larva per nymph, every three days, between the first and the final moult.

The nymphs reared collectively consumed on the average a smaller number of larvae than those reared individually. It may be that the habit of co-operation among individuals, during feeding, eliminated waste of food and enabled the nymphs to mature on a smaller number of larvae, than was possible in the individual rearings. Table XII summarizes the observations on food consumption, in the collective rearings.

TABLE XII  
SUMMARY OF FOOD CONSUMPTION IN THE COLLECTIVE REARINGS

	Lot number	2nd instar	3rd instar	4th instar	5th instar	Total, 4 instars
Average number of larvae consumed by one individual	A	1½	1	4	4½	10½
	B	1½	3½	2½	4	11

Four nymphs reared solely on animal food consumed a greater number of larvae than those provided with both animal and vegetable food. In these experiments, fall webworm larvae were used, and it was found advisable to disable them by piercing the thorax with a dissecting needle, or by crushing the thorax with forceps, so that they could not hopelessly entangle the *Podisus* nymphs in their webs. The greater food consumption of these nymphs may be due to the greater ease with which they obtained their food. The survival of these nymphs proved their independence of vegetable food, during the last four instars, provided enough insect food is available. The data obtained in these rearings are summarized in Table XIII.

TABLE XIII

NUMBER OF DISABLED FALL WEBWORM LARVAE CONSUMED BY NYMPHS OF *P. serieventris*

	2nd instar	3rd instar	4th instar	5th instar
Maximum number of larvae consumed by one individual	8	14	11	9
Minimum number of larvae consumed by one individual	5	5	5	3
Average number consumed	6	9½	7½	5½

The greatest number of larvae consumed by one nymph during the last four instars was 34 larvae; the least number was 21; and the average number was 28½.

#### *Dependence of Nymphs on Animal Food*

A few experiments were carried out to discover whether nymphs, having fed on animal food during the early instars, could thereafter subsist on a vegetable diet alone. The nymphs were captured in the field at various times and provided both animal and vegetable food until the succeeding moult. After the moult, the nymphs were supplied with vegetable food only (alder, balsam, cherry or lilac). Only a limited number of nymphs were used, but in all cases death occurred before the final moult. In all but two instances, death occurred in the same instar in which the vegetable diet was begun. The two exceptions were nymphs which completed the fourth stage on a purely vegetable diet, but succumbed in the fifth stage. The number of days upon the vegetable diet ranged from 8 to 38, with an average of 18 days, before death eventually occurred. The normal mortality of the later stages in rearing vials was approximately 35%, so the total mortality of the experimental nymphs on a vegetable diet can hardly be attributed to an unfavorable environment. It appears that *Podisus serieventris* nymphs of the later stages require animal food in order to develop to maturity.

*Feeding of New Adults*

Twenty-six adults were kept under observation from the final moult until their death. Of these, ten males and ten females were paired, each pair being placed in a separate vial, and six adults were placed individually in vials. Foliage and fall webworm larvae were supplied, the larvae being replaced as devoured, and the foliage renewed every few days. New adults were frequently observed sucking the juices from foliage, with living larvae untouched in the same vial.

In most cases, the adults did not begin to feed until a few days after the final moult. After feeding began, it was continued fairly regularly until the middle of September; after that, very little feeding was done. Field work ceased September 18, and thereafter the adults were kept indoors, the last individuals dying October 3. The total number of larvae consumed by the adults reared individually are shown in Table XIV.

TABLE XIV

TOTAL NUMBER OF LARVAE CONSUMED BY ADULTS OF *P. serieventris* REARED INDIVIDUALLY

Experimental number	C	D	E	F	G	H
Sex	Female	Male	Male	Female	Female	Female
Total number of larvae consumed	8	24	13	13	16	7

NOTE:—Average number for the six adults,  $13\frac{1}{2}$ .

The food consumption of the adults reared in pairs was relatively less than that of the adults reared individually. This may have been due to the co-operation among individuals, as was suggested for the nymphs. The total numbers of larvae consumed by the pairs of adults are summarized in Table XV.

TABLE XV

TOTAL NUMBER OF LARVAE CONSUMED BY PAIRS OF ADULTS OF *P. serieventris*

Experimental number	I	J	K	L	M	N	O	P	Q	R
Total number of larvae consumed	48	26	16	25	19	24	24	19	15	10
Average number of larvae per adult	24	13	8	$12\frac{1}{2}$	$9\frac{1}{2}$	12	12	$9\frac{1}{2}$	$7\frac{1}{2}$	5

NOTE:—Average number, for the 20 adults,  $11\frac{1}{2}$ .

The results of a small series of experiments indicated the dependence of adults on animal food; plant food was apparently insufficient to sustain life for long. The average adult life of 5 individuals deprived of all food after the final

moult was 15 days. The average adult life of 3 individuals supplied plant food was 23 days. And the average adult life of the 26 individuals supplied both animal and vegetable food was over 63 days.

### *Process of Attack and Feeding*

That *Podisus serieventris* is a capable predator is evidenced by the following observations. A second-stage nymph was found attached by its mouth parts to the genitalia of an adult cercopid, *Aphrophora parallela* (Say), in flight at the time of capture. Adult bugs were observed feeding on the moths of *Peronea variana* and *Ellopiia fiscellaria*, and on an adult plecopteran. Very small nymphs can overcome caterpillars many times their size.

The younger nymphs (second and third instars) are usually more active in their search and more aggressive in their attack, than are the older nymphs and adults, which usually capture their prey by stealth. Cleansing of the beak and antennae was frequently observed just prior to an attack, though the same operations were occasionally performed independently of an attack. In the process, the beak is extended, and the anterior legs are raised to grip the apex, one on each side; a transparent liquid may be ejected from the apex of the beak, and bathed over the tarsal segments and the distal portions of the tibiae. The tibiae are pressed against the sides of the beak, and the legs straightened; the tibiae are thus pressed along the beak apically. This may be repeated several times. The apical segments of the antennae are cleaned in a similar manner. Loosening of the setae in the labial groove is corrected by forcing the spurs of the anterior tibiae along the groove, distally.

In the approach to a victim, the extended antennae commonly are in a state of rapid vibration. The beak is held tip down, until close; here the bug usually stops, sets its legs, and makes contact with its victim by "leaning" forward. There is no definite selection of a soft area for the insertion of the setae; any part of the body wall, a proleg, the clypeus, the vertex or other sclerotized parts are successfully pierced. After the setae are inserted, there usually follows a terrific struggle, the victim thrashing about, often attempting to crush the beak with its mandibles. In most cases, attempts to gain release are futile.

The means by which the bug retains its grip has been questioned. The beak itself has no gripping function, but merely holds the setae together. The mandibular setae are rather coarsely barbed, and are forced into the tissues to a depth equal to about one-third the last labial segment. The maxillary setae are sharply pointed, without barbs; they are not forced deep into the tissues until the actual feeding begins. It has been the general opinion that the mandibular setae secure the prey. Baker (1) discredits this explanation and describes a reciprocating action of the setae, which have a strong tendency to curl inwards and backwards when released from the restraining pressure of the opposite seta of the pair, and suggests that the curling of the extended setae serves to secure the victim.

The prey becomes feeble in 3-15 min. after the setae are inserted, and the point of insertion is usually swollen and discolored. When the struggling



is over, the maxillary setae are thrust deep into the body; this is accomplished by the complete flexion of the first and second labial segments. The rapid probing movements of the setae are readily observed in *Peronea variana*, which is light green in color, with few hairs. There are occasional short periods of rest, possibly to allow the withdrawal of the larval juices. When one part of the body is emptied, the integument collapses, and the adjacent intact tissues are drawn within reach of the setae, to be broken down and pumped out. A peculiar pumping action, marked by alternate lifting and lowering of the antennae, is characteristic of feeding on animal food; this is less noticeable when the bugs feed on plant juices.

Second-stage *Peronea* larvae were sucked in 10 min., fully grown *Peronea* larvae in two to three hours. Nymphs, and adults as well, frequently co-operate in sucking from the same victim, in rearing cages.

In sucking plant juices the beak is applied more or less perpendicularly to the plant surface, and the setae extend only a short distance into the tissues. When feeding from deciduous foliage, the setae are frequently inserted in the larger veins on the lower surface.

### *Poisoning*

Predacious pentatomids have been suspected of utilizing a poisonous secretion by numerous investigators, including Muir and Kershaw (13), McDermott (9), Baker (1), and Tothill, Taylor and Paine (18).

Several observations suggested the presence of such a secretion in *Podisus serieventris*. (1) Small globules of a transparent liquid were observed on the tips of the beaks, after unsuccessful attacks upon caterpillars. (2) Caterpillars were frequently found dead in the rearing vials, without having lost their body juices. (3) Victims usually ceased their struggles soon after being pierced, before *Podisus* started to withdraw their juices. (4) Swelling and discoloration usually marked the point of insertion of the setae.

To test the possible occurrence of a toxic substance in *Podisus serieventris*, a number of larvae of different species were introduced into vials containing nymphs and adults. The bugs were allowed to attack the larvae and maintain their grip for a short period; they were then disengaged by means of a pencil or forceps. The larvae were isolated in vials with foliage, and their subsequent behavior noted.

Eighteen larvae were exposed to attack, for 3-90 sec. Twelve larvae died about an hour, or longer, after the attack. The six larvae that survived suffered some discomfiture, evidenced by feebleness and cessation of feeding. Death of the 12 larvae was not due to mechanical injury, because larvae pierced through the thorax with a dissecting needle resumed feeding after a day, and survived. This form of injury was much more severe than the mechanical injury caused by the setae of *Podisus*.

It seems probable, therefore, that *Podisus serieventris* is supplied with a toxic substance, presumably a secretion of the salivary glands, which nearly always causes local irritation, very often causes general feebleness, and in a

considerable number of cases causes death. Its effects may be correlated with the duration of the attack, but this was not determined.

### Host References

Insects preyed upon by *Podisus serieventris* are enumerated below; the list includes species referred to in the literature, and species which came under the writer's observation. To avoid unnecessary detail, the authorities for the various host species are not included, and the species names are transcribed as they appear in the literature. Species which were killed by *P. serieventris* in confinement are followed by the letter *c*, and those species which came under the writer's observation are followed by the letter *a*. All of the latter refer to Cape Breton, with the exception of *Diprion polytomum* Hartig, the record of which refers to Gaspé, Quebec, 1931 and 1932; and *Fenusa pumila* Klug, which refers to Fredericton, N.B., 1932, reported by Mr. C. C. Smith.

### Lepidoptera.

- Gelechiidae : *Recurvaria* sp. (probably *gibsonella*) (*a*) (*c*).
- Tortricidae : *Peronea variaria* (*a*).
- Geometridae : *Ellopiia fiscellaria* (*a*); *Nematocampa limbata* (*a*) (*c*);  
*Paleacrita vernata*.
- Notodontidae : *Datana ministra* (*c*).
- Lymantriidae : *Euproctis chrysorrhea*; *Notolophus antiqua*;  
*Orgyia leucostigma*; *Porthetria dispar*.
- Noctuidae : *Noctua c-nigrum* (*c*); *Pyrophila pyramidoides*.  
*Rhynchagrotis alternata*.
- Arctiidae : *Ilyphantria cunea*.
- Citheroniidae : *Anisota senatoria* (*c*); *Dryocampa rubicunda* (*c*).
- Saturniidae : *Attacus cecropia* (*c*); *Attacus promethea*; *Telea polyphemus* (*c*).
- Lasiocampidae : *Clisiocampa americana*; *Clisiocampa disstria*;  
*Tolyte velleda*.
- Nymphalidae : *Eu Vanessa antiopa*; *Limenitis ursula*.

### Coleoptera.

- Coccinellidae : *Adalia bipunctata*.

### Hemiptera.

- Pentatomidae : *Menecles insertus*; *Podisus cynicus*; *Podisus serieventris* (cannibalistic).

### Homoptera.

- Cercopidae : *Clastoptera* sp. (*a*) (*c*).
- Aphrophora parallela* (*a*).

### Hymenoptera.

- Cimbicidae : *Cimbex americana* (*c*).
- Tenthredinidae : *Diprion polytomum* (*a*).
- Fenusa pumila*.

In a recent paper, Plummer and Landis (14) reduced the number of *Epilachna corrupta* larvae, pupae and adults, consumed by various predators,

to an arbitrary basis. They state: "It was not always possible to supply a large number of individuals of this host of the same size and stage. For this reason it would be impossible to collate the value of the several species of predators without reducing the quantity of food consumed to some standard measure." An arbitrary value of unity was assigned to a third instar larva, and the other stages were evaluated in proportion to their relative weight. "By means of the values assigned above, 'food equivalents' have been determined from the actual laboratory data. A food equivalent may be defined as the quantity of food consumed by one individual in one day." This seems to be a useful method of accurately evaluating the food consumption of different species, of predators; but as an expression of the "value" of a predator, it seems to neglect the fact that it is not the weight of the insects, but the number of insects, destroyed, that is of real significance. The destruction of ten small larvae is certainly more significant, in terms of control, than the destruction of one full grown larva ten times the weight of the smaller ones. The distinction is submerged in reducing food consumption into "food equivalents."

### **Relation Between *Podisus Serieventris* Uhler and *Peronea Variana* Fernald, in Cape Breton\***

Reference to the epidemic of *Peronea variana* in Cape Breton has already been made in the introduction of this paper. It is not possible here to enter into a complete discussion of the biology of *Peronea*, but the more important points in its life history are outlined below.

#### *Life History of Peronea variana*

*Peronea variana* overwinters in the egg stage, the eggs being deposited during September on the needles of balsam fir, white spruce and to a less extent on red and black spruce. Hatching occurs during the last few days in May and the first few days in June. There are five larval stages. The young first-stage larvae burrow through the bud scales or crawl into the newly expanded shoots. Here the larvae feed during the first three stages, constructing small shelters of silk about themselves. As the new needles of balsam fir expand into a horizontal plane, and as defoliation progresses, there is a modification in the feeding habit, which generally affects the fourth- and fifth-stage larvae. Tubular, silken shelters are constructed along the stems, at the base of the new needles, which are devoured by the sheltered larvae. If defoliation is so severe that the new foliage is exhausted, the larvae move back and subsist on the old foliage.

Prior to pupation, which occurs from late in July to early in August, a more compact shelter is constructed among the excrement and dead needles; here the pupa transforms into the moth. The pupal stage ranges from 18 to 23 days. Moths are in flight during August and September, and oviposition is about finished by the end of September.

\* All data relating to the life history and population of *Peronea variana* Fernald have been obtained from the as yet unpublished 1930 and 1931 reports of the Division of Forest Insects, Dominion Entomological Branch.

### Stages Susceptible to Podisus Attack

Kirkland (7) records the sucking of gipsy moth eggs by the first stage nymphs of *Podisus serieventris*. Felt (5) states that *Podisus placidus* sucks the eggs of *Anisota senatoria*. It was not determined whether the eggs of *Peronea variana*, present during eight to nine months of the year, are actually sucked by *Podisus*. Numbers of perfectly formed, but empty and transparent egg shells were found in the spring of 1930; many of these showed no evidence of an emergence hole. Newly deposited *Peronea* eggs were placed in vials with adult bugs, but none were sucked after several days in the vials. It is therefore uncertain whether the empty transparent shells, found in the field, had been sucked by the bugs.

The first three larval instars are well protected in the compact new shoots. The fourth and fifth instars are more susceptible, and the pupal stage, with its long period of inactivity, is most open to attack. The adult moths are occasionally killed by *Podisus*, but the numbers thus destroyed are probably insignificant.

### *Podisus* as a Factor in the Outbreak

Examination of the area infested by *Peronea variana*, in August 1929, showed the outbreak to be widespread and the larvae to be fairly abundant. A high percentage of hatch was recorded in June 1930, and there was apparently low mortality among the early stage larvae. *Podisus* nymphs were found in fair numbers late in July, and the adults appeared early in August. The bugs took their toll of the budworms, but no estimate of the proportion of the larval population destroyed by *Podisus* in 1930 is possible. The larval population suffered a great reduction during the final stage, due to the operation of control factors other than *Podisus*; the number surviving to the pupal stage was about 200 for the average mature balsam tree in the area of medium infestation, and approximately 2000 for the average mature balsam in the heavily infested area. The activity of *Podisus* during the pupal stage of its host was evidenced by the occurrence of sucked pupae; these were devoid of contents, with the pupal skin intact. Results with four sample collections of pupae, relative to the control of the pupal stage by *Podisus* are shown in Table XVI.

TABLE XVI

PERCENTAGE OF BUDWORM LARVAE DESTROYED BY  
*P. serieventris*

Sample number	Locality	Number of pupae in sample	Percentage sucked by <i>Podisus</i>
1	St. Peters	301	14
2	Grand River	493	8 (approx.)
3	Grand River	1028	5
4	Louisburg	1263	8

In addition to empty intact pupal skins, another type was frequently found, containing the dead adult, or pre-imago. These may have been sucked by *Podisus*, or may have died from other cause. Because of this uncertainty, dead pupae of this kind were not included in the percentage shown above.

There was a heavy flight of moths in September 1930, but comparatively few eggs were laid. The egg population overwintering from 1930 to 1931 was at the approximate rate of 4000 to 6000 for the average mature balsam tree in the heavily infested area, only about one-tenth the egg population of the previous year in that area.

The eggs hatched late in May, and the first few days of June, 1931. The *Peronea* population suffered great reduction between the first of June and the tenth of July, when the young nymphs of the current generation of *Podisus* began to appear in numbers. The nymphs were most numerous the latter part of July, the population ranging from 14 to 35 individuals per mature balsam tree. The budworm population was at this time greatly depleted, due chiefly to parasites and a disease which was very prevalent. The flaccid bodies of dead budworms, whose death was attributed to predatism by *Podisus*, comprised as much as 30% of the *Peronea* population in one count made early in August; in other counts, few of the sucked remains were found. The difficulty of finding the dead bodies, many of which must have dropped from the trees, and of distinguishing between parasitized and sucked remains, made estimation of the control afforded by *Podisus* a matter of uncertainty. Dead flaccid budworms, however, comprised less than 10% of the larval population found on 12 trees, examined between July 10 and August 11.

The budworm population was almost wiped out by the operation of natural control factors, before the period of pupation arrived. Accordingly, the activity of *Podisus* at this time could not be evaluated in terms of sucked pupae. The *Peronea* outbreak was so reduced that the writer did not see a single moth in the balsam-spruce forests about Grand River, in 1931.

At the end of July, the *Podisus* population ranged from 14 to 35 individuals per mature balsam tree. Of 17 trees examined during August, the number of bugs per tree ranged between one and ten; and of four trees examined early in September, the greatest number of bugs per tree was four. Rearing experiments, already described, indicated the inability of *Podisus serieventris* to subsist for long on a purely vegetable diet. Presumably the heavy reduction in the *Peronea* population during the season caused a corresponding reduction in the *Podisus* population through starvation, for no other factors of natural control were found operating against *Podisus* in the Grand River forest.

The conclusions drawn from this study agree in essentials with those reached by Plummer and Landis (14), who, in regard to the relation between predacious insects and *Epilachna corrupta* Muls. in Mexico, state: "The peak of the larval population of the bean beetle is reached and damage to bean foliage accomplished before an appreciable number of pentatomids appear in the fields. As the winter survival of *E. corrupta* in most parts of the infested areas of Mexico is high, it is likely that the number of *E. corrupta* destroyed by predators during the late summer does not greatly reduce the number of beetles necessary to maintain the bean beetle the following season." The writer's observations on *P. serieventris* are also in agreement with the

general conclusion reached by Weiss (22); of predacious pentatomids, Weiss states they are "important in a limited way..... as a whole they lack elasticity, and do not, on account of their limited powers of reproduction, respond to any sudden increase in phytophagous forms. Beyond a certain point it is useless to expect more from them....." It is very probable that the *Podisus* population increased during 1929 and 1930 when *Peronea* was present in large numbers; but in 1931, the greatly reduced host population limited in a very definite manner the number of predators that were able to reach maturity. The value of *P. serieventris* as a predator of forest insects would be greatly increased if it were capable of maintaining its population, during the decline of its host, by subsisting on vegetable food.

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# STERILIZATION OF NARCISSUS BULBS BY IMMERSION IN SILVER NITRATE — POTASSIUM CYANIDE SOLUTION *IN VACUO*<sup>1</sup>

BY W. NEWTON<sup>2</sup>, R. J. HASTINGS<sup>3</sup> and J. E. BOSHER<sup>3</sup>

## Abstract

Through the use of a dye solution, evidence was obtained that a liquid disinfectant may be forced into the narcissus bulb parts invaded by nematodes and fly larvae by immersion *in vacuo*.

An investigation of the lethal properties of solutions against nematodes and their influence upon bulb growth led to the selection of a silver nitrate solution as a promising disinfectant, but owing to instability of silver nitrate in the presence of chlorides and other substances in tap water and in dirt clinging to bulbs, its use had no commercial possibilities. However when silver salt was combined with potassium cyanide in the ratio of 1 to 3 by weight, an effective solution of satisfactory stability was obtained.

A solution of silver nitrate 0.05% and potassium cyanide 0.15% by weight, forced into narcissus bulbs by an evacuation process, effectively destroyed bulb nematodes and bulb fly larvae without significant injury to bulb growth under greenhouse conditions.

Field tests with bulbs treated in silver nitrate-potassium cyanide solutions resulted in the reduction of infection from 26.8 to 1%, a 96% control, and no evidence of injury in the foliage or bloom was detected.

Little attention has been paid to the sterilization of narcissus bulbs with solutions of chemicals owing to the internal location of the principal parasites, the bulb nematode *Tylenchus dipsaci* Kuhn, and the larvae of the bulb fly *Merodon equestris* Fab. The ordinary immersion treatments had little effect, owing to the failure of the disinfectant to reach the bulb parts invaded by the parasites. Longford (1) has recently advocated a method of forcing the sterilizing liquid into the bulbs, by immersing the bulbs in a sealed container and by creating a vacuum over the immersed bulbs. He claimed that the subsequent release of the vacuum forced the sterilizing liquid, a formalin solution, into the bulbs. According to Mackie and Milbrath (2), this method of forcing sterilizing liquids into bulbs is not new. Apparently it was investigated in California in 1922, but was abandoned as having little promise. However, our investigations indicate that the vacuum treatment induces an effective penetration of the sterilizing fluids in the case of narcissus bulbs.

The hot water treatment as developed by Ramsbottom (3), Van Slogteren (4) and others, has been the only satisfactory commercial method of destroying the bulb nematode and the fly larvae in narcissus bulbs. With the object of developing an alternative method, sterilization by immersion in solutions of chemicals *in vacuo* has been investigated.

## Experimental

In the initial small-scale experiments a Fruehling and Schultz vacuum desiccator and a Nelson laboratory vacuum pump were used. The de-

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siccator held approximately 50 bulbs and 5 litres of solution and could be evacuated to 3 in. of mercury, in ten minutes. In the semicommercial-scale experiments a pressure cooker of 150-gallon capacity was used. It was equipped with a vacuum gauge and approximately 500 lb. of bulbs could be submerged in 100 gal. of solution therein. With the Nelson laboratory pump this container filled with submerged bulbs could be evacuated to 5 in. of mercury in 30 min. The release of a vacuum of 5 in. of mercury appeared to be sufficient to force the liquid into the tissue invaded by the nematode and bulb fly larvae. The penetration was studied by immersing the bulbs in a solution of methyl violet in the vacuum desiccator. Bulbs left in the solution for 20 min. after the release of the vacuum (total time of immersion 30 min.) were compared with a  $3\frac{1}{2}$ -hour ordinary immersion and the comparison is illustrated in Fig. 1, A and B. The difference in penetration was striking. The evacuation process forced the dye in between practically every scale from top to bottom and although the ordinary immersion was over a period seven times as long, sections of the immersed bulbs proved that there was little dye penetration.

### *Toxicity of Chemicals Towards Nematodes*

The tolerance of living nematodes to various chemicals was approximately determined by increasing the concentration of the chemical in a water suspension of living nematodes to the point where motility ceased within two hours. By this means and by observing the influence of the chemicals upon the subsequent growth of treated bulbs, the following chemicals were discarded as having little promise, because they were either non-toxic to the nematodes or were decidedly toxic to the bulb tissue at the lethal concentrations: ammonium oxalate, ammonium sulphate, boric acid, calcium chloride, copper acetate, copper sulphate, ferric sulphate, hexyl resorcinol, lead acetate, lime (chlorinated), malachite green, mercuric chloride, mercuric nitrate, phenol, potassium bisulphite, potassium chlorate, potassium cyanide, potassium dichromate, potassium polysulphide, sodium benzoate, sodium borate, sodium ferrocyanide, sodium fluoride, sodium thiosulphate, sulphite waste liquor, tannic acid, urea, and zinc chloride.

The following chemicals appeared to be lethal to nematodes at the recorded concentrations: acetic acid, 3.0; beechwood creosote, 0.5; chlorox (5% sodium hypochlorite), 10.0; formalin, 3.0; mercuric cyanide, 1.0; potassium cyanide, 0.3; potassium permanganate, 3.0; potassium bisulphite, 5.0; silver nitrate, 0.05; sodium nitrate, 5.0; sodium sulphide, 0.6%.

### *Pot Experiments in 1931*

Six of the most promising solutions were further tested. Lots of 25 bulbs were passed through three dilutions of each chemical and subsequently planted in six-inch pots in a greenhouse. The influence of each dilution was observed in the foliage, root and bloom development, as well as upon the parasitic nematodes and fly larvae. As a check, a representative lot was run



A.



B.

FIG. 1. Methyl violet penetration as influenced by the evacuation process and ordinary immersion. A. Evacuation process; B. Ordinary immersion.



through the standard hot water treatment for nematode control, namely, a three-hour immersion in a water bath held at 112°F. The results are given in Table I.

TABLE I

EFFECT OF CHEMICAL TREATMENT OF DISEASED NARCISSUS BULBS BY THE EVACUATION PROCESS ON THE DEVELOPMENT OF THE BULB AND THE NEMATODE PARASITES

Treatment	Plants with apparently normal			Plants in which nematodes survived %
	Foliage %	Root %	Bloom %	
Untreated	88	56	52	48
Hot water, 3 hr. 112°F.	84	84	40	36
Sodium sulphide 1.5%	40	44	32	0
1.0%	55	65	45	20
0.5%	84	76	60	40
Chlorox (5% NaOCl) 16%	75	52	48	32
Silver nitrate 0.15%	68	64	52	0
0.10%	75	75	65	0
0.05%	80	88	64	4
Potassium cyanide 1.5%	8	0	0	Bulbs decayed
1.0%	40	36	16	4
0.5%	56	56	44	32
Formalin 2.0%	24	16	20	8
1.0%	15	15	15	20
0.5%	32	36	20	24
Acetic acid 1.5%	20	5	10	Bulbs decayed

The results suggested that silver nitrate at 0.05% to 0.15% was a satisfactory lethal agent for nematodes in narcissus bulbs. The other five chemicals either failed to destroy the nematodes or were extremely toxic to the host bulbs.

#### *The Instability of Silver Nitrate*

The instability of silver nitrate in a solution proved to be the limiting factor in the commercial adoption of this chemical as a disinfectant. The original results could be duplicated only when clean bulbs were used and when the silver nitrate was dissolved in water low in chlorides. When a lot of dirty bulbs were run through the semicommercial set-up, the silver salt rapidly and completely disappeared from the solution and the immersion *in vacuo* was not effective at the intermediate concentration, namely 0.1%.

In order to prevent the silver from being thrown out of solution as insoluble chlorides and other insoluble compounds through the interaction of the silver nitrate with the salts normally present in the dirt on the bulbs and in tap water, a combination of silver nitrate and potassium cyanide was used in the ratio of one to three by weight. In this solution the silver is present largely

as part of the complex  $\text{Ag}(\text{CN})_2^-$  ion and silver in this form is not thrown out of solution by chlorides. The large excess of potassium cyanide ensures stability even when a considerable part of the cyanide is lost through reactions with the dirt on the bulbs.

The solution adopted as a standard contained silver nitrate 0.05 and potassium cyanide 0.15% ( $\frac{1}{4}$  and  $1\frac{1}{4}$  lb. respectively, in 100 gal. of water). By adding soil to this solution in quantities much in excess of what would normally come in contact with the solution through the treatment of dirty bulbs, the loss of silver was insignificant. In practice about one-tenth of the original volume is lost when each lot of wet bulbs is taken from the silver nitrate-potassium cyanide solution. The replacement of this volume with fresh solution of standard strength is all that appears necessary to maintain an effective concentration.

Four lots of abnormally dirty bulbs were put through the same solution in succession adding only sufficient fresh solution to maintain the original volume. The kill of both nematodes and bulb fly larvae appeared to be as effective in the last lot as in the first. A fraction of the bulbs so treated were examined 14 days after the treatment, and no living nematodes or bulb fly larvae were found.

A special lot of bulbs containing fly larvae (*Merodon equestris*) were put through the standard silver nitrate-potassium cyanide evacuation process, and the larvae were examined by Mr. W. Downes\*. He found that the treatment effected a 100% mortality.

Bulbs treated by immersion in solutions of chemicals *in vacuo*, like those treated with hot water, should be thoroughly drained and partly dried before planting in heavy or wet soils, or injury may follow through oxygen deficiency.

### Field Experiments

Nematode-infested King Alfred bulbs supplied by a commercial grower were used in the field plot experiments. After treatment they were planted in  $\frac{1}{100}$ -acre plots and trenches were dug between the plots to prevent the migration of the nematodes from one plot to another under distinctive treatment. The data are presented in Tables II and III.

The silver nitrate-potassium cyanide solutions at standard, two and three times standard strength markedly reduced the amount of nematode infection compared with the untreated as shown by the reduction in the number of plants bearing "spikkels". However, these solutions do not appear to be quite as effective as hot water. The apparent perfect control by the hot water may be due to the presence of 0.5% formalin or to the fact that the temperature was maintained at 114°F., two degrees higher than standard. Previous experiments showed that the King Alfred variety is more tolerant to high temperatures than most varieties of narcissus. As expected, silver

\*The authors are pleased to acknowledge the assistance of Mr. W. Downes, Dominion Entomological Branch, Victoria, B.C.

TABLE II

NEMATODE CONTROL AS EFFECTED BY FORCING SOLUTIONS INTO THE BULBS BY IMMERSION *in vacuo* AND BY A MODIFIED HOT WATER TREATMENT

Treatment	Time of treatment; weeks after lifting	Bulbs planted (King Alfred)	No. and percent with "spikkels"		Control relative to untreated
		Number	No.	%	%
Untreated		250	67	26.8	
Silver nitrate 0.1%	2	236	17	7.1	
	3	250	19	7.6	
	4	236	21	8.9	
	5	250	9	3.6	
	7	250	9	3.6	
		1222	75	Av. 6.1	77
Potassium cyanide 0.5%	2	125	15	12.0	
	3	118	14	11.8	
	4	125	26	20.4	
	5	125	10	8.0	
		493	65	Av. 13.2	50
Silver nitrate 0.5% Potassium cyanide .15%	7	100	1	1.0	
Silver nitrate 0.1% Potassium cyanide 0.3%	7	900	6	0.6	
Silver nitrate 0.15% Potassium cyanide 0.45%	7	200	5	2.5	
		1200	12	Av. 1.0	96
Sodium sulphide 1.0%	2	125	20	16.0	
	3	119	32	26.8	
	4	125	35	28.0	
	5	125	31	24.0	
		494	118	Av. 23.7	11
Hot water and 0.5% formalin, 3 hr. at 114°F.	3½	250	0	0	100

nitrate alone gave some measure of control, for clean bulbs were used, but the silver nitrate solution was not as effective as when combined with potassium cyanide, and no other solution under test gave promise.

To determine whether any deterioration of the silver nitrate-potassium cyanide solution took place, a series of 14 lots of uncleaned bulbs, a total of 700, were run in succession through five litres of solution adding only enough fresh solution to compensate for the loss of liquid absorbed by the bulbs. A total of three litres of solution was absorbed. The results are given in

Table III, and indicate that the efficiency of the solution remains constant when only sufficient fresh solution is added to maintain the necessary volume to cover the bulbs.

TABLE III

THE INFLUENCE OF SUCCESSIVE IMMERSION OF BULB LOTS IN THE SAME SOLUTION OF SILVER NITRATE-POTASSIUM CYANIDE

Chemical solution	Sequence of lots	Number of bulbs treated	Number of plants with spikkels	Per cent diseased
Check—untreated		250	67	26.8
Silver nitrate 0.1% and potassium cyanide 0.3%	1st	50	0	0
	2nd	50	1	2
	3rd	50	0	0
	4th	50	0	0
	5th	50	0	0
	6th	50	0	0
	7th	50	0	0
	8th	50	0	0
	9th	50	0	0
	10th	50	0	0
	11th	50	1	2
	12th	50	0	0
	13th	50	1	2
	14th	50	0	0
		700	3	Av. 0.4

NOTE:—A vacuum desiccator was used for this experiment and it was necessary only to supplement the initial five litres of solution with one litre after the 7th, 9th and 12th lots, to keep the bulbs covered.

No evidence of injury to the bulbs could be detected as induced by the silver nitrate-potassium cyanide solution at standard, two and three times standard concentrations. The measurements of the foliage and bloom showed that in these experiments there was no significant difference between the plants from treated bulbs and the healthy plants in the untreated plots. The only difference noticed was in the case of bulbs in an advanced state of decay through nematode infestation. Untreated, such bulbs would produce a feeble growth but after treatment they would not grow at all.

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# NEMATODE INFESTATION SYMPTOMS ON BARLEY AS A MEANS OF DETERMINING THE EFFICIENCY OF CHEMICALS AS LETHAL AGENTS AGAINST *TYLENCHUS* *DIPSACI* KUHN<sup>1</sup>

BY W. NEWTON,<sup>2</sup> R. J. HASTINGS<sup>3</sup> AND J. E. BOSHER<sup>3</sup>

## Abstract

Barley is suggested as a detector crop for the presence of living nematodes, *Tylenchus dipsaci*, in soil, owing to rapid development of nematode disease symptoms on barley.

A satisfactory source of inoculum consists of the white masses of coiled nematodes that can be seen when the basal plate is removed from badly diseased narcissus bulbs. These masses remain viable for six months or longer when removed from the bulbs.

Low temperatures and high light conditions favor the development of the nematode disease symptoms in barley seedlings, after nematodes are transferred from narcissus bulb to autoclaved soil planted with barley. Such barley seedlings were broad-leaved and stocky. Under low light and high temperatures, conditions that favor the development of spindly seedlings, the nematode disease symptoms are inconspicuous or absent.

Few chemicals appear to be lethal to the bulb nematode. Of 100 tested only phenol, silver nitrate, and potassium or sodium bisulphite were lethal at dilute concentrations.

In a recent paper (1) the writers reported that when barley, wheat and oats are planted in pots in soil inoculated with the narcissus bulb nematode *Tylenchus dipsaci* Kuhn, conspicuous symptoms of the nematode disease rapidly developed in the seedlings. The writers have since found that nematode infestation symptoms develop in fall rye seedlings when likewise inoculated. Owing to the fact that the symptoms were most conspicuous in the barley seedlings it was thought that after treatment with various chemicals the presence of living nematodes might be conveniently determined by transferring the nematode suspensions to pots of soil planted with barley. Barley has many advantages over narcissus bulbs in the detection of living nematodes. In contrast with narcissus bulbs the chances are small that barley seed is ever naturally infected with the narcissus bulb nematode. Furthermore, the cost is much lower and data can be obtained in a much shorter time. One month from the time the barley is planted the symptoms of nematode infection are usually evident. Moreover, unlike the use of narcissus bulbs the use of barley enables the experimenter to conduct a series of experiments regardless of season, for crops of barley seedlings can be grown in rapid succession throughout the year.

<sup>1</sup> Manuscript received May 8, 1933.

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### *Inoculum*

Macerated tissue of soft bulbs severely infested with *Tylenchus dipsaci* were used in the preliminary experiments as the source of inoculum. This inoculum proved to be unsatisfactory owing to the frequent transfers of *Fusarium* species and other parasites that cause damping off of the barley seedlings. In these experiments practically pure cultures of nematodes were used. The inoculum was obtained by selecting severely infected bulbs that had been kept in storage for at least two months. These bulbs were soft and partly decayed and when the basal plates were removed, white cottony masses (Fig. 1) of what proved to be thousands of coiled dormant nematodes (Fig. 2) could be seen with the naked eye. These masses were pricked off and maintained as inoculum, and when stored in cork-stoppered bottles for six months the nematodes therefrom would revive in about three hours when suspended in tap water. A needle transfer from the stock inoculum usually yielded over 1,000 nematodes. These, revived in water, were used throughout these experiments and were found capable of infecting barley, wheat, oat and rye seedlings as well as disease-free narcissus bulbs.

### *Symptoms of Nematode Attack on Barley Seedlings*

The symptoms of nematode infestation on barley seedlings are easy to recognize. The leaves show definite white spots, mainly along the midrib (Fig. 3).<sup>\*</sup> Sometimes these are slightly raised, and occasionally a leaf is markedly deformed. Infestation shows up early, the symptoms usually being conspicuous four weeks after seeding.

## Experimental

### CONDITIONS FAVORING INFESTATION AND DEVELOPMENT OF SYMPTOMS

Barley seeds were sown thickly in five-inch pots containing steam-sterilized soil (1 hr. at 25 lb pressure), and were inoculated with 100 cc. of water containing over 1,000 nematodes. The pot cultures were then allowed to develop in an ordinary greenhouse, each lot under distinct but variable conditions of light and temperature. Observations were made when the seedlings had reached 8 to 16 cm. in height. The conditions of growth and the results are given in Table I.

Nematode-infestation symptoms are conspicuous in barley seedlings grown under an environment that favors the development of stocky plants with broad fully expanded leaves, *e g*, full light and low temperatures. Under such an environment the symptoms can be readily detected four weeks after seeding when the plants are approximately 10 cm. high. When the seedlings are grown in the semi-shade or diffused light at low temperatures or under rapid growing conditions induced by high temperatures, spindly plants develop whereon the symptoms of nematode infestation are seldom found.

*See also Reference 1, Fig. 1.*



FIG. 1. Bulbs with nematode clusters in the basal plate and on the old roots. FIG. 2. Part of a cluster of nematodes in a drop of water  $\times 50$ . FIG. 3. Barley seedlings showing symptoms of infection by the bulb nematode.



TABLE I

THE PERCENTAGE OF BARLEY PLANTS INFESTED AS INFLUENCED BY THE ENVIRONMENT OR SEEDLING TYPE

Date of experiment and source of inoculum	Environment and seedling type	Type of plant			Per- centage infected
		Av. height of plants, cm.	Av. length of stem, cm.	Av. width of leaves, cm.	
Oct. 13-Nov. 2 KING ALFRED BULBS Saanichton, B.C.	Pots in unheated green- house, temperature range 43-89° F. full light: stocky plants	12	2 0	0 7	17.7
Nov. 17-Dec. 2 SIR WATKINS BULBS Gordon Head, B.C.	Pots in closed case in green- house, temperature range 60-85° F. diffused light. spindly plants	15	4 0	0 4	1.3
Jan. 3-Jan. 29 SIR WATKINS BULBS Gordon Head, B.C.	Pots in unheated chamber off greenhouse, temper- ature range 35-85° F.— A. Full light: stocky plants	10	1 5	0 7	20.5
	B. Semi-shade, pots rest- ing on boards: spindly plants	15	4 5	0 5	13.3
	C. Semi-shade, pots plunged in peat: spindly plants	15 5	4 5	0 4	6.6
Feb. 3-Feb. 28 PRINCEPS BULBS Colwood, B.C.	Pots in unheated chamber off greenhouse, temper- ature range 32-80° F. full light: stocky plants	9 0	1 0	0 6	56.0

## TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS DETERMINED BY THE BARLEY INDEX METHOD

By studying the distribution of a dye solution the writers recently concluded that solutions of chemicals can be forced into the regions of the narcissus bulbs inhabited by the bulb nematode by immersion *in vacuo*. Furthermore, it was found that immersion *in vacuo* in a combination solution of potassium cyanide and silver nitrate was a promising control for the bulb nematode (4). The present study is part of a search for agents lethal to nematodes and relatively non-toxic to plant tissue at the lethal concentrations. The standard hot water treatment for bulb sterilization, in vogue for 15 years, has lately been observed to yield inconsistent results. The treatment, it will be recalled, as developed by Ramsbottom (5) required a three hours' submergence in hot water at 110° F., and a retest of this treatment under precise control has shown that nematodes were alive in old lesions when the bulbs were examined in the spring. There appears to be an urgent need for a modification of the treatment. Raising the temperature of the hot water is effective in ensuring a more complete destruction of the nematodes, but the risk to the health of

the bulb is often great. An alternative is to add a supplemental killing agent to the hot water, but the present knowledge of lethal agents for the bulb nematode is very limited. Apart from the writers' report that immersion in silver nitrate-potassium cyanide solution *in vacuo* is effective, they can find reference to only one other chemical agent: *viz.*, formalin. This was recommended by Longford (3) as a cold dip applied *in vacuo*, and by the United States Department of Agriculture (6, 7) as a supplement to the standard hot water treatment, to prevent the survival of nematodes that escape into the hot water. However, Johnson and Godfrey (2) report chloropicrin as an effective soil fumigant against Root Knot, hence this chemical might find application as an agent for the destruction of the bulb nematode.

### Experimental

The toxicity of the following chemicals was determined in the manner outlined above, except that the nematodes were suspended in a chemical solution instead of water. If they were destroyed by the chemical there could, of course, be no infestation. On the other hand, if the chemical were not lethal, the nematodes would probably be as capable of infecting barley as when suspended in water. The tests were made in triplicate. One cubic centimetre of each prepared solution was placed in a test tube. A needle transfer of the nematodes was made from the stock inoculum into each of these test tubes, and after contact with the solution for two hours each test tube was filled with water and emptied into a three-inch pot of sterilized soil previously seeded to barley. The pots were then thoroughly watered and kept under the optimum conditions for infection as described above. The observations were recorded from three to four weeks after seeding and are presented as Table II.

TABLE II

TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS REVEALED BY THE ABILITY OF THE IMMERSSED NEMATODES TO INFECT BARLEY

Chemical	Concentration				Chemical	Concentration			
	Satur- ated solution	2%	1%	0.5%		Satur- ated solution	2%	1%	0.5%
1. Acetamide		N			11. Ammonium carbonate		N		
2. Acid benzoic	N				12. Ammonium chloride		N		
3. Acid boric		X			13. Ammonium citrate		N	N	N
4. Acid carbolic (phenol)		O	O	O	14. Ammonium oxalate		N		
5. Acid molybdic		N			15. Ammonium phosphate (secondary)		N		
6. Acid oxalic		N			16. Ammonium sulphate		N		
7. Acid picric	N				17. Ammonium tartrate		N	N	N
8. Acid pyrogalllic		N			18. Amyl acetate	N			
9. Acid salicylic	N				19. Barium chloride		N	N	N
10. Acid sulphanilic	N								

*Symbols*—N, plants infected—solution non-toxic to nematodes.

X, chemicals toxic to barley, indefinite results.

O, healthy plants, solution lethal to nematodes.

TABLE II—*Concluded*

TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS REVEALED BY THE ABILITY OF THE IMMERSED NEMATODES TO INFECT BARLEY

Chemical	Concentration				Chemical	Concentration			
	Satur- ated solu- tion	2%	1%	0.5%		Satur- ated solu- tion	2%	1%	0.5%
20. Brucine	N				62. Potassium phosphate (secondary)		N	N	N
21. Cadmium sulphate		N			63. Potassium phosphate (tertiary)		N	N	N
22. Cresol		N			64. Potassium persulphate		N		
23. Creosote	N				65. Potassium silver cyanide		O		
24. Cupric acetate		N	N	N	66. Potassium sulphate		N		
25. Cupric nitrate		O	N	N	67. Potassium thiocyanate		N		
26. Cupric sulphate		O	N	N	68. Quinine	N			
27. Diphenylamine	N				69. Resorcinol		N		
28. Dyes—Auramine O.S.		N			70. Saponin		N		
29. Fluoresceine					71. Silver nitrate		N	O	O
30. Indian blue		N			72. Sodium acetate		O		
31. 2 R.S.		N			73. Sodium benzoate		N		
32. Magenta P.S.		N			74. Sodium bicarbonate		N		
33. Malachite green		N			75. Sodium bisulphite		O	O	O
34. Rhodamine		N			76. Sodium borate		N		
35. Ethyl acetate		N			77. Sodium carbonate		N		
36. Ferric ammonium sul- phate		O	O	N	78. Sodium chloride		N		
37. Ferric chloride		N			79. Sodium ferrocyanide		N		
38. Ferrous ammonium sul- phate		N			80. Sodium fluoride		N		
39. Ferrous sulphate		N			81. Sodium fluosilicate (Con- solidated Mining and Smelting Co.)	N			
40. Formalin		O	N	N	82. Sodium fluosilicate (Vir- ginia-Carolina Chemi- cal Co.)	N			
41. Glycerine		N			83. Sodium hydroxide		O		
42. Hydrochinone		N			84. Sodium nitrite		N		
43. Lead acetate		N			85. Sodium nitrop.usside		N		
44. Lead carbonate	N				86. Sodium oxalate		N		
45. Magnesium phosphate	N				87. Sodium peroxide		O		
46. Magnesium sulphate		N			88. Sodium potassium tartrate		N		
47. Mercuric chloride		O	O	N	89. Sodium phosphate		N		
48. Mercuric cyanide		X			90. Sodium salicylate		X		
49. Mercuric nitrate		O	N	N	91. Sodium sulphate		N		
50. Naphthylamine, alpha	N				92. Sodium sulphite		N		
51. Naphthol, beta	N				93. Sodium thiocyanate		N		
52. Piperine	N				94. Sodium thiosulphate		N		
53. Potassium acetate		N	N	N	95. Stannous chloride		N		
54. Potassium bichromate		N			96. Thymol	N			
55. Potassium bisulphite		O	O	O	97. Urea		N		
56. Potassium chlorate		X			98. Vanillin		N		
57. Potassium nitrate		N			99. Zinc chloride		N		
58. Potassium iodate		X			100. Cheshunt Compound		O	N	N
59. Chinosol		N							
60. Potassium permanganate		N	N	N					
61. Potassium phosphate (primary)		N	O	N					

The most promising chemicals appeared to be phenol, potassium and sodium bisulphite and silver nitrate. In general, the work revealed that the bulb nematodes are difficult to destroy by chemicals, even by substances commonly used as fungicides and insecticides. An experimental search for lethal agents is under way among the less common chemicals.

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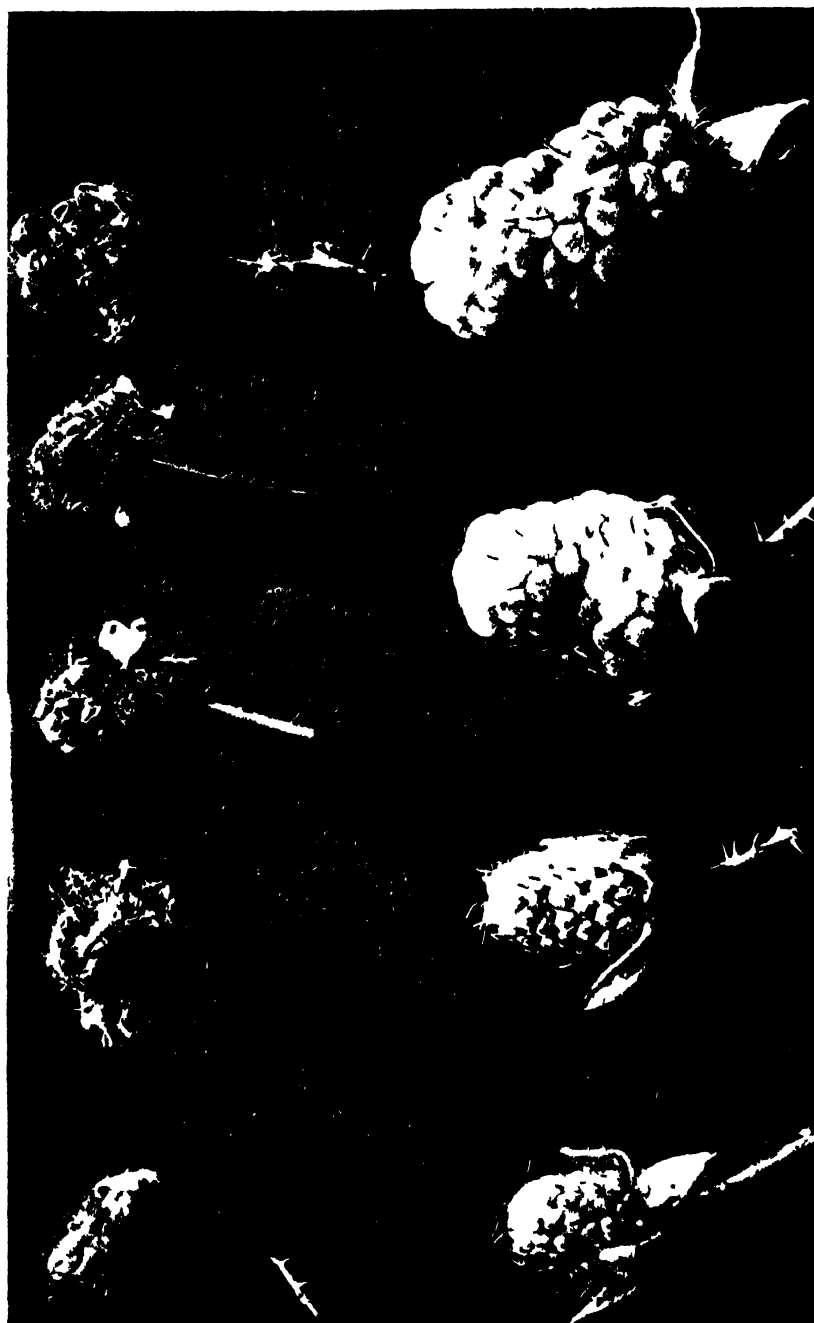


FIG 1 Disease of loganberry caused by *Haplospora deformans* Syd Upper—Diseased Lower—healthy

# ANTHER AND STIGMA BLIGHT OF LOGANBERRY<sup>1</sup>

BY J. DEARNESS<sup>2</sup> AND W. R. FOSTER<sup>3</sup>

## Abstract

A new disease of loganberry, an anther and stigma blight, is reported from British Columbia. This disease is caused by *Hapalosphaeria deformans* Syd., a new fungus for North America. The fungus prevents pollination of a number of the drupelets and a deformation of the fruit results.

## Introduction

In the spring of 1932, the junior author's attention was directed to deformed loganberries with a white fungus on the anthers and stigmas (Fig. 1). The fungus was also found on the petals which were touching the anthers. The estimated loss from the fungus in a number of fields in Royal Oak and Gordon Head districts, Vancouver Island, was 3 to 5%. Growers were alarmed enough to send reports and samples of the injury to the laboratory. The authors collaborated in a study of the fungus.

## Etiology

It was found that Sydow (2) described the hyphomycetous form of this fungus with superficial mycelium and isthmus-connected globoid conidia on *Rubus caesius* L. in 1907 and named it *Paepalopsis deformans* Syd. In the following year Diedicke studied the disease further and inferred that the *Paepalopsis* in this case is a stage or form of a sphaeropsis fungus found chiefly in the anthers. Sydow concurred in this inference. Diedicke (1) expressed the surprise both of himself and Sydow that the presumptive hyphomycete is also a good sphaeropsis. Upon the discovery, Sydow founded a new genus which he called *Hapalosphaeria* on the type species *H. deformans* Syd.

The fungus in the distorted flowers of the loganberry, *Rubus loganobaccus* Bailey agrees with the description of the above named species. Both the curious hyphal form and the sphaeropsis were present in at least one opening flower bud examined by the senior author. In one anther there were no fewer than 56 pycnidia each with its multitude of smooth, colorless globoid spores 3-4  $\mu$  in diameter.

The fungus prevents the pollination of a number of the drupelets and a deformation of the fruit results, as shown in Fig. 1. The presence of the disease in the old world and its first reported appearance on the Pacific Coast of America give ground for the supposition that it is an Asiatic parasite of the raspberry which has invaded this continent by the Northwest.

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<sup>1</sup> Manuscript received April 10, 1933.

<sup>2</sup> Contribution from the Provincial Laboratory of Plant Pathology, Saanichton, British Columbia, Canada, with financial assistance from the National Research Council of Canada.

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## CHEESE-RIPENING STUDIES<sup>1</sup>

### Casein-splitting Abilities of Lactic Acid Bacteria

BY BLYTHE ALFRED EAGLES<sup>2</sup> AND WILFRID SADLER<sup>3</sup>

#### Abstract

The casein-splitting ability of each of seven cultures isolated from Kingston cheese has been studied. The cultures are Gram positive non-gelatin-liquefying coccus forms; some appear as chains in young milk culture, others as pairs. The methods of Orla-Jensen and Wasteneys and Borsook were used.

It is shown that: (a) Cultures EMB<sub>2</sub> 166 and 168 fail to attack casein; (b) the casein-splitting abilities of cultures EMB<sub>1</sub> 131, 133, 173, 195 and EMB<sub>2</sub> 173 are established and well-defined; (c) the type of proteolysis characteristic of cultures EMB<sub>1</sub> 173 and 195 leads to the formation of large amounts of the simpler degradation products, whereas the proteolytic breakdown characteristic of cultures EMB<sub>1</sub> 131, 133 and EMB<sub>2</sub> 173 is not as complete; (d) culture EMB<sub>2</sub> 173 is distinct in its casein-splitting abilities, as is evident when the results obtained by the two methods are compared; (e) unable to attack casein, cultures EMB<sub>2</sub> 166 and 168 attack the non-protein-nitrogenous components of milk; (d) culture EMB<sub>2</sub> 166 may be capable of protein synthesis.

It has been shown by Orla-Jensen (4) in his studies on the lactic acid bacteria that the casein-splitting ability of an organism may be defined by determining the soluble nitrogen and amino nitrogen after incubation of the culture in chalk milk at the appropriate temperature for a period of six weeks. From his work it is to be seen that some organisms fail to hydrolyze the milk protein; that certain organisms produce an appreciable amount of soluble nitrogen, but amino nitrogen in a limited measure only; and that certain organisms attack the milk protein in such manner that not only is a considerable amount of soluble nitrogen formed, but also a relatively large amount of amino nitrogen. Not only have the writers employed the methods of Orla-Jensen in determining the casein-splitting abilities of lactic acid bacteria but also have used the method of Wasteneys and Borsook (9)—the method developed by them for the fractional analysis of incomplete protein hydrolysates.

#### Methods

For the determination of total nitrogen, soluble nitrogen and amino nitrogen after the manner of Orla-Jensen the writers have followed the methods used by him in his studies on the lactic acid bacteria (1, 4, 5, 6). In determining the proteose nitrogen, peptone nitrogen and subpeptone nitrogen after the manner of Wasteneys and Borsook the procedure has been almost identical with that described by the writers (1).

<sup>1</sup> Manuscript received May 1, 1933.

These studies were provided for by a Research Fund established jointly by the Empire Marketing Board and the University of British Columbia. As from Feb. 1 to Sept. 30, 1933, work proceeds under a joint grant of the Empire Marketing Board and the National Research Council of Canada, laboratory facilities being found by the University of British Columbia.

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In the work on certain cultures it was found impossible to filter the solution when following the procedure for determining protease, because large quantities of what is presumably a soap salted out. Consequently in working on these cultures one departure from the technique has had to be made. The solution was filtered after it had stood for 15 min.—instead of for one hour, the length of time defined in the original method (9)—two grams of anhydrous sodium sulphate was added to the filtrate and the mixture was then kept at 35° C. for 45 min. before filtering in a water-jacketed filter maintained at 33° C.

### Experimental

Employing the methods cited (1, 4, 5, 6, 9), a study of the casein-splitting abilities of seven lactic acid producing coccus forms has been undertaken. All the strains were recovered from Kingston cheese (7). Cultures EMB<sub>1</sub> 131, 133, 173 and 195 were isolated from a Kingston cheese of the "make" of Dec. 4, 1929—, the time the cheese was two weeks old and characteristic of the type (7). Cultures EMB<sub>2</sub> 166, 168 and 173 were isolated from another Kingston cheese— the make of Dec. 4, 1929—at the time the cheese was two months old (7). The seven cultures are Gram positive non-gelatin-liquefying coccus forms: some appear as chains in young milk culture and some are to be seen as pairs: the total titratable acidity produced by each strain in milk and in milk enriched with yeast extract has been determined repeatedly (7). The influence of defined nitrogen sources on the sugar-fermenting abilities of certain of these cultures has been studied (7, 8) and the effect on their sugar-fermenting abilities of enriching peptic casein digest broth with different concentrations of yeast extract has been determined (7).

The results of the determinations of the casein-splitting abilities of the seven cultures are given below in Table I.

### Discussion

It is to be seen that there is considerable variation in the total nitrogen content of the milk employed as controls for the determinations.

In the case of the controls the soluble nitrogen figure is always considerably higher than is the non-protein-nitrogen figure (Table I). Consequently, inasmuch as the trichloroacetic acid filtrate is made up of non-protein-nitrogenous compounds only, that amount of nitrogen obtained on subtraction of the non-protein-nitrogen figure from the corresponding soluble nitrogen figure, represents "something" of a greater complexity than is protease, "something" of a complexity approaching that of a complete protein.

It may be suggested that this "something" present in the control milk—milk autoclaved with calcium carbonate—is formed by the autoclaving process from a "something" physically more simple to be found in fresh skim milk.

Enquiring into the validity of this hypothesis, a quantity of fresh skim milk was divided into three equal parts: one aliquot remained untreated; the other two portions were autoclaved at 14 lb. pressure for 25 min. after the



addition of 3% calcium carbonate to one of them. The soluble nitrogen, the nitrogen of the trichloroacetic acid filtrate direct and the non-protein-nitrogen respectively in each aliquot was then determined. The results are given, in mgm. per 100 cc. milk, in Table II.

TABLE II

Milk	Soluble nitrogen	Non-protein nitrogen	Nitrogen of trichloroacetic filtrate direct
Milk—fresh	74.00	33.80	68.80
Milk—autoclaved	63.00	36.20	38.80
Milk—autoclaved with CaCO <sub>3</sub>	68.00	35.04	39.60

The soluble nitrogen figures agree rather closely and the non-protein-nitrogen figures approximate the one the other, Table II. It is equally clear that the latter are considerably lower than the former. The figures for the nitrogen determinations on the trichloroacetic acid filtrate direct—that is the filtrate prior to concentration—agree closely with the non-protein-nitrogen figures in the case of milk autoclaved with or without calcium carbonate. On the other hand, in the case of fresh skim milk the nitrogen content of the trichloroacetic filtrate direct is quite closely comparable with the soluble nitrogen figure.

It would appear that in being subjected to the autoclaving process the proteins in milk assume a complexity of a nature comparable with the complexity arrived at when subjected to heat as part of the Wasteneys and Borsook procedure for the complete removal of protein by trichloroacetic acid.

Whilst it is shown that cultures EMB<sub>2</sub> 166 and 168 fail to attack casein, the casein-splitting abilities of cultures EMB<sub>1</sub> 131, 133, 173, 195 and EMB<sub>2</sub> 173 are established and well defined, Table I.

When the amount of amino nitrogen formed by an organism is taken as an indication of the extent to which the breakdown of casein has been carried, it is shown that the type of proteolysis characteristic of cultures EMB<sub>1</sub> 173 and 195 is of a nature leading to the formation of large amounts of the simpler degradation products and that for cultures EMB<sub>1</sub> 131, 133 and EMB<sub>2</sub> 173 the proteolytic breakdown is not as complete, (Table I).

Applying the method of Wasteneys and Borsook, it is shown that a more detailed defining of the nature and amount of the protein decomposition products—proteose, peptone and subpeptone—in the control milk and in the milk in which the organisms have been grown is obtained. When this method is applied, the striking difference, shown above, in the nature of the proteolytic breakdown brought about by cultures EMB<sub>1</sub> 131 and 133 on the one hand and by cultures EMB<sub>1</sub> 173 and 195 on the other hand cannot be seen.

Culture EMB<sub>2</sub> 173 would appear to be distinct among the casein-splitting strains reported upon. While the amount of soluble nitrogen formed by the

culture is comparable with the amount of soluble nitrogen formed by cultures EMB<sub>1</sub> 131, 133, 173 and 195, it is shown that the amount of amino nitrogen formed by culture EMB<sub>2</sub> 173 is not comparable with the amount of amino nitrogen formed by any of the other casein-splitting strains—actually the amino nitrogen figure being lower than the amino nitrogen figure of the control, (Table I). The non-protein nitrogen formed approximates the soluble nitrogen formed when the casein-splitting abilities of cultures EMB<sub>1</sub> 131, 133, 173 and 195 are considered, whereas in the case of culture EMB<sub>2</sub> 173 the non-protein nitrogen formed is considerably less than is the amount of soluble nitrogen formed, the major portion of the non-protein nitrogen consisting of proteose nitrogen—the more complex of the protein-breakdown entities. Thus the distinct nature of the proteolytic ability of culture EMB<sub>2</sub> 173 is the more clearly seen when the results obtained by employing the method of Orla-Jensen are compared with the results obtained by using the method of Wasteneys and Borsook, (Table I). If we may interpret the results of the determination of the distribution of protein and protein-breakdown products respectively as an expression of the protein hydrolysis-protein synthesis equilibrium attained by an organism, may it not be that the amount of nitrogen obtained on subtraction of the non-protein-nitrogen figure from the soluble nitrogen figure in the case of culture EMB<sub>2</sub> 173 represents “something” of a greater complexity than is proteose, “something” of a complexity approaching that of a complete protein. In so far as its proteolytic ability is concerned culture EMB<sub>2</sub> 173 would appear to be quite distinct as compared with the strictly non-casein-splitting forms on the one hand and the active casein-splitting forms on the other hand.

When the results obtained by the method of Wasteneys and Borsook for the non-casein-splitting strains are considered it is shown that cultures EMB<sub>2</sub> 166 and 168 are capable of acting upon the non-protein-nitrogenous components of milk and the results obtained for culture EMB<sub>2</sub> 168 appear to suggest that the nature of this action is dependent upon the kind of nitrogen moieties to be found in a particular milk (Table I). Further, it is to be seen that for culture EMB<sub>2</sub> 166, the non-protein-nitrogen figure is lower than is the non-protein-nitrogen figure of the control, a finding which would seem to indicate protein-synthesizing action—an hypothesis already proposed by Hucker (3) when working with certain lactic-acid-producing coccus forms.

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# THE EFFECT OF RADIANT ENERGY ON DIASTASE ACTIVITY<sup>1</sup>

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## Abstract

The effects of radiant energy as procured from a mercury arc, using full irradiation of varying intensity and using also individual wave-lengths transmitted by a monochromatic illuminator, have been determined. The enzymes investigated are the diastases (amylases) of saliva and of malt and the production, first of erythroextrin and second, of maltose from starch, is used as an indicator of the enzyme activity. These two phases are designated the dextrinogenic and the saccharogenic. Full irradiation retards the dextrinogenic and the saccharogenic activity of both salivary and malt diastase in an inverse relation to intensity. In the case of salivary diastase the rates of dextrin production and of maltose production are decreased by the green and the far ultra-violet wave-lengths, while both tend toward stimulation when irradiated with the red yellow and near ultra-violet wave-lengths. The monochromatic effects on malt diastase are generally inhibitory for the dextrinogenic phase and stimulatory for the saccharogenic phase. These results may be explained by the presence of two enzymes constituting the diastase, one dextrinogenic, the other saccharogenic; either may be the less active and so become the "pace setter" for maltose production; in the dextrinogenic phase one only is considered, in the saccharogenic phase, both are involved; in salivary diastase the dextrinogenic enzyme is the "pace setter", while in malt diastase the saccharogenic enzyme is usually the "pace setter"; full illumination, however, retards the dextrinogenic enzyme until it becomes the "pace setter". The effects of monochromatic light on the growth of *paramecium* parallel the effects on the activity of salivary diastase and the effects of monochromatic light on the sporulation of *Colletotrichum* parallel the effects on the saccharogenic activity of malt diastase.

## Introduction

There is evidence from results obtained by the authors in their previous work (6, 7, 8, 9), and from the experiments of other investigators, that light has a profound effect on both plant and animal protoplasm and that this effect must be based on one or more basic factors. What these factors are, has yet to be determined. Several theories, however, have been tentatively propounded.

According to F. L. Gates (2) the effect of measured monochromatic ultra-violet energy used to kill bacteria shows characteristic and similar curves at each wave-length, but an appreciable amount of energy must be incident on the bacteria before any of them succumb, and widely different intensities of energy are required to produce these curves at different wave-lengths. The reciprocals of these curves are similar to the absorption curves of certain derivatives of the nucleoproteins which are related to cell growth and reproduction. This conclusion is supported by the known fact that the active agent of chicken tumors is associated with a nucleoprotein, which, in all cases of sarcomatous cells, gives a positive Feulgen reaction. It is therefore possible that the lethal effect and the inhibition of cell division caused by ultra-violet light may be related to its action on the nucleoproteins of the nucleus.

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F. I. Harris and H. S. Hoyt (5) suggest the possibility that the susceptibility of protoplasm to ultra-violet light is conditioned by the selective absorption of the toxic rays by the aromatic amino acid radicals of the proteins.

The authors have suggested (6) that resonance phenomena may be involved, and that certain particles in a colloidal or other state possess vibration periods such that their movements are affected by the specific frequencies of the radiant energy. On this basis subsequent effects may differ from the initial ones owing to associated changes in the colloidal condition.

As there is a certain dependence of enzyme activity on the colloidal state it was thought that the effect of radiant energy on enzyme action might throw some light on the nature of the basic factors causing inhibitory or stimulatory effects on protoplasmic activity.

### Review of Literature

Light influences enzyme actions by destroying the enzyme itself or by modifying its activity. Lockemann, Thies and Wichern (11) found that the inhibitory effect of diffused light on peroxidase and blood catalase was in the following order: white, blue, red and darkness. Reinle (13) found that the methylene-blue-formaldehyde-reductase reaction of cow's milk was decreased by the action of ultra-violet light. Getchell and Walton (3) state that ultra-violet light is more potent in the destruction of peroxidase activity than X-rays, radium or visible light.

Pincussen (12) found that the action of light on diastase and urease is dependent on the dilution of the enzyme, the various impurities present and upon the type of the reaction. Radium radiations were also found to be injurious to rennet but to have a stimulative effect on diastase. X-rays also have varied effects on enzymes, the diastatic activity of urine, serum and various fluids being unchanged while that of pepsin and amylase was considerably altered.

H. J. Fuller (1) experimented with plant tissue injured by ultra-violet radiation in respect to catalase and diastase activity and found that the activity of these enzymes actually increased to a considerable extent in tissues exposed to destructive wave-lengths and that there was no evidence whatever of a diminution of enzyme activity, a condition contrary to the results of previous investigators. This author believes that the injurious effects of ultra-violet light must be traceable to some physiological derangement other than the inactivation of enzymes.

It would seem that various forms of radiant energy have varying effects on enzymes from different sources, and the work reported here is an attempt to find some correlation between the effect of light on plant enzymes and on animal enzymes in the hope that some definite organization of the facts may be developed, and that some connection may be established between these results and those previously reported on the effect of ultra-violet light on growth and reproduction.

### Experimental

The most widely distributed enzymes that control the hydrolysis of the higher carbohydrates are the diastases. These enzymes hydrolyze carbohydrates to an end-product of achroödextrins and maltose with the intermediate production of erythrodextrins. In these experiments the animal amylase used was ptyalin, the diastatic enzyme present in human saliva. The plant enzyme used was malt diastase from sprouted barley.

The divergence in results obtained during the hydrolysis of starch by diastase of various origins has led to a belief in the presence of two enzymes, a starch liquefying enzyme and a saccharogenic enzyme. In this paper the former is designated as "dextrinogenic". Sherman and Schlesinger (14) and Kendall and Sherman (10) have demonstrated that the liquefying action is predominant in pancreatic amylase and the saccharifying in malt diastase, and have also shown that the liquefying enzyme is predominant in salivary diastase. These workers found that the proportion of the two actions was usually constant for each particular enzyme although great differences in proportion were noticeable in enzymes of varied sources, this difference being ascribed "to the presence of complements or activators which are required for the splitting up of certain definite atomic groups".

It was thought that light, having a specific effect on the action of enzymes, might have a different effect on the two enzymes present in diastase, acting as an inhibitor or activator of the different groups. The experiments here described were therefore carried out on the dextrinogenic action of diastases from various sources as distinct from the saccharogenic action.

#### A. SALIVARY DIASTASE

##### *Materials and Methods*

Samples of saliva were obtained in the following manner. The mouth was washed out with distilled water at 40° C.; 20 cc. of water was then held in the mouth for one minute and collected. This was repeated twice. The 40 cc. was then shaken, filtered and the resulting solution kept in the dark. It was found that the activity of the solution became less on the third day, so a fresh solution was made up every three days and was found to be remarkably constant in its activity. The achromic point of the saliva was ascertained and if it varied greatly from four minutes, adjustment was made of the concentration of the solution to obtain an achromic point of constant value.

Soluble starch, c.p., was used as the substrate throughout all the experiments.

Solutions of starch were made up of the following concentrations: 0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5%.

In order to ensure a pH maximum of 6.7, 25 cc. of phosphate buffer of pH 6.7 was used, that pH being the optimum condition for ptyalin activity. Twenty-five cc. of 1% sodium chloride was also added as the action of animal amylase has been proved to be conditioned by the presence of neutral salts in quantities varying from 0.02 to 2.0%.

The 0.1% starch solution would therefore be made up as follows: 5% starch solution, 4 cc.; buffer, 25 cc.; 1% sodium chloride, 25 cc.; distilled water, 146 cc. The other solutions were made up with the required proportions of starch and distilled water.

### Procedure

Enzyme solution (3 cc.) was placed in each of two quartz tubes. One tube was fitted into the slit of the monochromatic illuminator or irradiated directly in front of the quartz mercury lamp and the other was kept in the dark as a control.

At the completion of irradiation, 1 cc. of the irradiated solution was diluted to 100 cc. with distilled water and 1 cc. of this solution placed in each of the series of test tubes containing 5 cc. of the graded starch mixtures. Thus 0.01 cc. of the original plus 5 cc. of starch solution was present in each test tube. The same process was repeated for the control. One drop of toluene was added to each test tube, which was then shaken and placed in an oven at 38–40° C. At stated intervals of time one drop was taken from each of the series of test tubes, irradiated and control, placed on a glass plate, using a dropping pipette, and one drop of *N*/10 iodine added. The first appearance of red coloration, denoting conversion of starch to erythrodestrins, was noted and the number of the test tube recorded.

To ascertain the saccharogenic activity of the salivary enzyme, one drop of the irradiated solution was added by means of a dropping pipette to each of two test tubes containing 5 cc. of 4% starch solution. At the same time one drop of the control solution, which had been kept in diffused light in the laboratory, was placed in each of the control test tubes and the entire series placed in the oven at 38–40° C.

After a period of 24 hr. the amount of sugar reduced in the irradiated and control tubes was estimated by means of the Wood-Ost copper carbonate method of sugar estimation (16).

At first readings were taken after six hours' incubation, but the results were found in most cases to be small, the 24-hr. readings giving a more easily recorded result.

### Results

#### 1. *The Effect of Full Illumination from a Quartz Mercury Lamp on the Dextrinogenic Action of Saliva*

(a) Three cc. of enzyme were irradiated at various distances from a quartz mercury lamp for a period of one hour and the achromic point of the solution ascertained (Table I).

TABLE I

THE EFFECT OF IRRADIATION ON THE SACCHAROGENIC ACTIVITY OF SALIVA

Distance from light source, cm.	30	20	10	7	5	3
Achromic point (time taken to convert starch to achroödextrin and maltose), min.	4	6	12	36	64	No end-point

These results show that the activity of the enzyme is greatly reduced by the action of light in direct relation to the distance from the source.

(b) Three-cc. samples of salivary diastase were irradiated for one hour at distances of 10, 7, 5 and 3 cm. from the source of light. The effect of the various irradiated enzyme solutions on the series of starch tubes, as previously described, was ascertained at varying periods of time, and a comparison made with the control (Table II, Fig. 1).

TABLE II

THE EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE DEXTRINOGENIC ACTIVITY OF SALIVARY DIASTASE

(Activity of enzyme expressed as percentage concentration of starch solution hydrolyzed to erythrodextrin in a measured time)

Distance from light source, cm.	Duration of digestion in hours							
	1	2	3	6	10	24	48	96
Control	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0
10	.5	.75	1.0	1.5	2.0	2.5	3.0	4.0
7	.2	—	.5	1.0	1.5	2.0	2.5	3.0
5	.2	—	.3	.5	.75	1.0	1.5	2.0
3	.1	—	—	.2	—	—	—	—

It will be seen that the full light from a mercury arc lamp has a decided inhibitory action on the activity of salivary diastase, evidently in varying proportion to the intensity of the light and the time. The rise in temperature of the extracts so irradiated was negligible as compared with the control, except in the case of irradiation at a distance of 3 cm. from the source of light, when the temperature was raised about 3° C. above room temperature. The almost total inactivation of the enzyme so irradiated may be therefore partly due to a possible destructive action of the wave-lengths of the infra-red part of the spectrum. Similar results were obtained when the saccharogenic activity was measured after an identical series of irradiations.

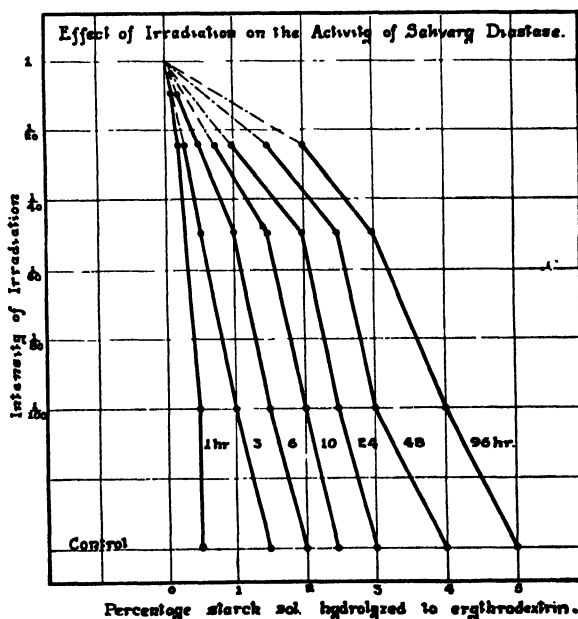


FIG. 1. The effect of radiant energy of various intensities upon the rate of salivary diastase activity, as measured by the erythrodextrin produced.

The experiments to determine the effect of full irradiation at different distances, as compared with a control kept in room light, show that the magnitude of the effect varies with the intensity, under these conditions. The curves (Fig. 1) representing the percentage of starch hydrolyzed to erythrodextrin after exposure to various intensities, regulated by distance from the light source, show that the inhibiting effect increases at a greater rate than the reciprocal of the square of the distance. The intensity of the light at the distances indicated has not been determined directly but it is presumed that in this case the intensity varies as the reciprocal of the square of the distance. It would appear that the inhibiting effect is proportionately greater at higher intensities. The marked similarity of the curves representing the percentages of starch hydrolyzed after irradiation during the time periods, 1, 3, 6, 10, 24, 48 and 96 hr. indicates that the effect of irradiation upon the enzyme endures and is constant for a period of four days after the experimental exposures to radiant energy.

*The Specific Effect of Monochromatic Light on the Dextrinogenic and Saccharogenic Activities of Salivary Diastase (Tables III and IV. Figs. 2, 3 and 4)*  
**Class 1. Wave-lengths having no dextrinogenic effect.** The wave-lengths  $\lambda$  6152 Å,  $\lambda$  5819 Å and  $\lambda$  3821 Å seem to exert no appreciable effect on the dextrinogenic action of the diastase used, whereas  $\lambda$  6152 Å and  $\lambda$  3821 Å exert a minimal stimulating effect, and  $\lambda$  5819 Å a greater stimulating effect, on the saccharogenic activity of the diastase.

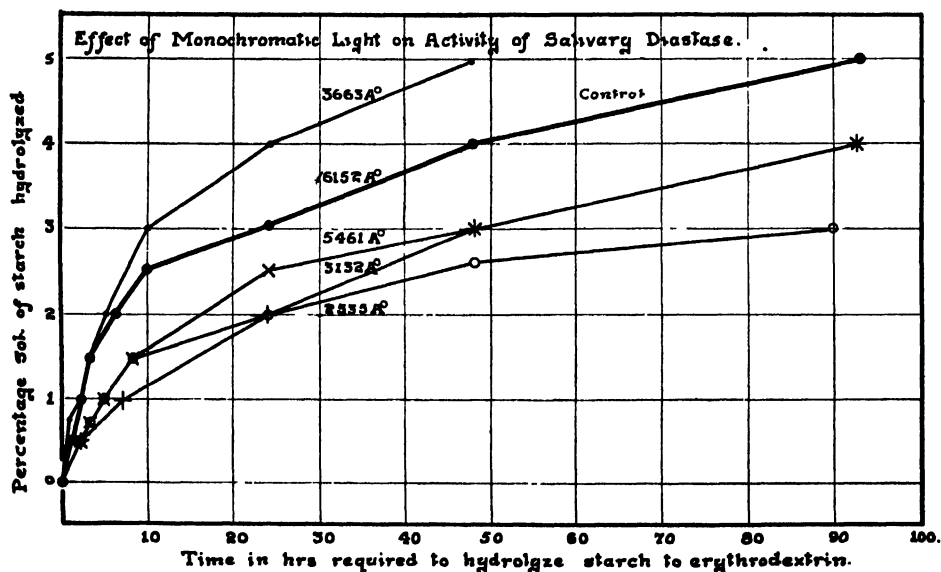


FIG. 2. The effect of specific light wave-lengths of the mercury arc spectrum on the dextrinogenic activity of salivary diastase.

**Class 2. Wave-lengths having a stimulating effect.**  $\lambda$  3663 Å is apparently the only line exerting stimulation on both saccharogenic and dextrinogenic activities, whereas  $\lambda$  6152 Å,  $\lambda$  5819 Å,  $\lambda$  5461 Å and  $\lambda$  3821 Å stimulate the

sugar forming portion of the diastase and  $\lambda$  4359 Å and  $\lambda$  4078 Å give a slight initial stimulation of the dextrin producing portion.

TABLE III

THE SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE DEXTRINOGENIC ACTIVITY OF SALIVARY DIASTASE

(Activity of the enzyme expressed as percentage concentration of starch solution hydrolyzed to erythrodextrin in a given time. Enzyme irradiated for 24 hours)

Wave-length	Hours of digestion													Total*	Diff.**
	1	2	3	4	5	6	7	8	9	10	24	48	96		
Control	.5	1.0	1.5	—	—	2.0	—	—	—	2.5	3.0	4.0	5.0	15.0	—
6152 Å	.5	1.0	1.5	—	—	2.0	—	—	—	2.5	3.0	4.0	5.0	15.0	0.0
5819 Å	.5	1.0	1.5	—	—	2.0	—	—	—	2.5	3.0	4.0	5.0	15.0	0.0
5461 Å	.3	.5	.75	—	1.0	—	—	1.5	—	—	2.5	3.0	4.0	11.05	-3.95
4916 Å	.3	.5	.75	1.0	—	1.5	—	—	—	2.0	2.5	3.0	4.0	11.05	-3.95
4359 Å	.75	1.0	1.5	—	2.0	—	—	—	—	2.0	3.0	4.0	5.0	15.25	0.25
4078 Å	.75	1.0	1.5	—	—	2.0	—	—	—	2.0	3.0	4.0	5.0	15.25	0.25
3821 Å	.5	1.0	1.5	—	—	2.0	—	—	—	2.5	3.0	4.0	5.0	15.00	0.0
3663 Å	.5	1.0	1.5	—	2.0	—	—	—	—	3.0	4.0	5.0	5.0	17.00	2.0
3132 Å	.3	.3	.5	—	.75	—	1.0	—	—	—	2.0	3.0	4.0	10.10	-4.9
3022 Å	.3	.5	.75	—	—	1.0	—	—	—	—	3.0	4.0	4.0	12.55	-2.45
2967 Å	.3	.5	.75	—	—	1.0	—	—	—	—	2.0	3.0	4.0	10.55	-4.45
2804 Å	.3	.5	.75	—	—	—	1.0	—	—	—	2.5	3.0	4.0	11.05	-3.95
2700 Å	.5	.5	.75	—	—	1.0	—	—	—	—	2.0	3.0	4.0	10.75	-4.25
2535 Å	.3	.5	.75	—	—	1.0	—	—	1.5	—	2.0	2.5	3.0	9.05	-5.95

\*Total—Sum of data for 1, 2, 3, 24, 48 and 96 hr.

\*\*Diff.—Difference between the activity of the control and of the irradiated enzyme.

TABLE IV

EFFECT OF MONOCHROMATIC LIGHT ON THE SACCHAROGENIC ACTIVITY OF SALIVARY DIASTASE  
(Readings expressed as mgm. maltose present after 24 hours' digestion)

Wave-length	Control	Irradiated	% Difference	Mean effect
6152 Å	50 64	50 67	0 4.8	2.4
5819 Å	58 62.5	65 69	13.8 10.5	12.12
5461 Å	57 59	60 60.5	2.5 5.2	3.85
4916 Å	52 57.5	42.5 45.5	18.2 20.8	-19.5
4359 Å	56 55	50 49	10.7 10.9	-10.6
4078 Å	50 49	35 37	30.0 24.4	-27.2
3821 Å	42.5	44	3.5	3.5
3663 Å	53 45	55 48	3.7 6.6	5.1
3132 Å	61 55	55 48	9.8 12.7	-11.2
3022 Å	69 66	55 58	16.8 13.6	-15.2
2967 Å	52 56	45 42	13.4 25.0	-19.2
2804 Å	53 57.5	42 45	17.0 21.7	-19.3

*Class 3. Wave-lengths exerting an inhibitory effect.*  $\lambda$  5461 Å,  $\lambda$  4359 Å,  $\lambda$  4078 Å and the ultra-violet mercury lines used,  $\lambda$  3132 Å,  $\lambda$  3022 Å,  $\lambda$  2967 Å and  $\lambda$  2800 Å, caused a decided inhibition of the dextrinogenic activity. Of these,  $\lambda$  5461 Å gives a slight saccharogenic stimulation. In no case is the activity entirely destroyed by monochromatic light of the low intensity used.

### General

It would seem from these results (Fig. 3) that the specific effects of the monochromatic light of the far ultra-violet spectrum (*i.e.*, beyond  $\lambda$  3663 Å) are very similar for all phases of diastatic action in saliva, and that these

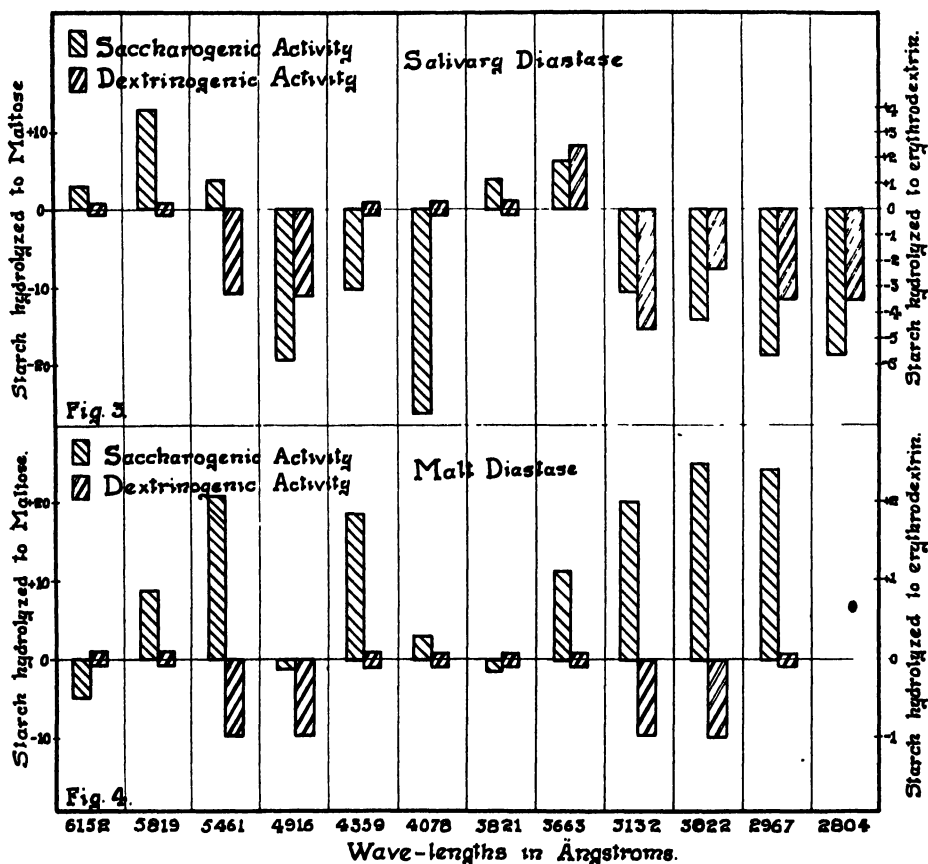


FIG. 3. A graph to compare the specific effects of various light wave-lengths on the dextrinogenic and the saccharogenic phases of hydrolysis by salivary diastase.

FIG. 4. A graph to compare the specific effects of various light wave-lengths on the dextrinogenic and the saccharogenic phases of hydrolysis by malt diastase.

effects are decidedly inhibitory and cause a reduction in the rate of the reaction. The near ultra-violet ( $\lambda$  3821 Å,  $\lambda$  3663 Å) spectrum seems to cause a slight stimulation which changes to decided inhibition for all phases as the green portion of the spectrum is approached. Stimulation again characterizes

the yellow-red end of the visible spectrum;  $\lambda$  5461 Å, being transitional, is the only line showing contrary effects for dextrinogenic and saccharogenic activity. It would seem, however, that the saccharogenic portion of the salivary diastase is more readily affected by irradiation, stimulation and inhibition being reported for lines of the spectrum which cause slight or undetermined effects on the dextrinogenic portion of the enzyme.

#### *The Time Factor in Relation to the Light Effect on Diastase*

The time-hydrolysis curve of the control (Fig. 2) illustrates the relatively rapid rate of digestion during the early period and the decreasing rate as the time increases. At 3 hr. the 1.5% starch solution is hydrolyzed to erythro-dextrin; at 10 hr. the 2.5% solution is hydrolyzed and at 96 hr. the 5.0%. Other experiments (15) have shown that an optimum concentration of substrate characterizes such reactions, but the results cannot be explained on this basis unless the optimum is below 0.3% starch solution. Generally the curves showing either retardation ( $\lambda$  5461 Å and  $\lambda$  2535 Å) or stimulation ( $\lambda$  3663 Å) show a type of time-rate curve similar to that of the control. On the contrary,  $\lambda$  3132 Å causes a primary retardation which is relatively greater, while  $\lambda$  4359 Å and  $\lambda$  4078 Å cause a primary stimulation for one hour followed by an absence of appreciable effect. Again, it would appear that with slight exception the modification of the enzyme induced by irradiation persists during the four-day period of its subsequent activity.

#### B. MALT DIASTASE

Malt diastase (0.75 gm.) was shaken for 15 min. in 250 cc. of distilled water and then filtered. This solution, plus two drops of toluene as a preservative, was kept in the dark in a stoppered bottle.

The same concentrations of starch solutions were made up as were used for the salivary series, but the pH was kept between 4.8 and 5.2 by means of a phthalate buffer. The 0.1% starch solutions were therefore made up as follows:—5% starch solution, 4 cc.; buffer, 25 cc.; distilled water, 171 cc.

Enzyme solutions (3 cc.) were placed in each of two quartz tubes and irradiated as in the previous experiment.

To ascertain the dextrinogenic action of the diastase three drops of the irradiated malt diastase was added by means of a dropping pipette to the starch series with two drops of toluene per test tube, shaken, and placed in the oven at 38–40° C. Three drops of the non-irradiated control was also added to another series and that in turn placed in the oven.

A record was made of the test tube in which the first appearance of red coloration, denoting conversion of starch to erythro-dextrin, was noted.

To ascertain the saccharogenic activity of the enzyme the same procedure was followed as for salivary diastase, except that the amount of sugar reduced was ascertained after only six hours' incubation, the saccharogenic action in this case being more rapid than is that of the salivary diastase.

The amount of maltose produced was measured by means of the Wood-Ost copper carbonate method of sugar estimation (16).



**Results****1. Effect of Full Illumination from a Quartz Mercury Lamp on the Activity of Malt Diastase**

A decided inhibitory effect was recorded, both for the dextrinogenic and sugar-reducing activities, increasing with the intensity of the light, the greatest inhibition being found when the solutions were irradiated at 3 cm. distance from the source of light (Tables V and VI).

TABLE V

EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE DEXTRINOGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Distance from source, cm.	Days of digestion											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.5	2.0	—	2.5	—	—	3.0	—	—	4.0	—	—
10	1.0	1.5	—	—	—	2.5	—	—	—	3.0	—	—
7	1.0	1.5	—	—	2.0	—	—	—	2.5	3.0	—	—
5	.75	1.0	—	—	1.5	—	—	—	—	2.5	—	—
3	.3	.5	—	.75	—	1.0	—	—	—	1.5	—	—

TABLE VI

EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE SACCHAROGENIC ACTIVITY OF MALT DIASTASE

(Readings expressed as mgm. maltose present after 6 hours' digestion)

Distance from source, cm.	Exp.	%	Exp.	%	Exp.	%	Av. %
Control	12.5	100	31	100	22	100	100
10	6.0	48	16	51	10	45	46
5	5.5	44	12	39	8.5	40	41
3	4.0	32	8	26	7.0	32	30

TABLE VII

SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE DEXTRINOGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Wave-length	Days of digestion											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.5	2.0	—	2.5	—	—	3.0	—	—	4.0	—	—
5461 Å	1.5	—	—	2.0	—	—	2.5	—	—	3.0	—	—
4916 Å	1.5	—	—	—	2.0	—	2.5	—	—	3.0	—	4.0
3132 Å	1.5	—	2.0	—	—	—	2.5	—	—	3.0	—	4.0
3022 Å	1.5	—	—	2.0	—	—	—	2.5	—	3.0	—	4.0

NOTE:—Wave-lengths 6152 Å, 5819 Å, 4359 Å, 4078 Å, 3821 Å, 3663 Å, 2967 Å, 2804 Å, 2700 Å, 2535 Å showed no effect (same as control).

2. *The Specific Effects of Monochromatic Light on:—*

*A. The dextrinogenic activity of malt diastase (Table VII).* It was found that only four wave-lengths, *i.e.*,  $\lambda$  5461 Å,  $\lambda$  4916 Å,  $\lambda$  3132 Å and  $\lambda$  3022 Å, had any appreciable effect on the diastatic action of the malt solution. The lack of effect in the shorter wave-lengths may be due largely to the fact that the intensity of the incident light was not sufficient to initiate any change in enzyme action, although it must be noted that  $\lambda$  4916 Å,  $\lambda$  3132 Å and  $\lambda$  3022 Å are all lines of very low intensity, as compared with  $\lambda$  5461 Å, and all exert a decidedly inhibitory action, pointing to the fact that intensity is not the controlling factor in this case.

*B. The saccharogenic activity of malt diastase (Table VIII).*  $\lambda$  6152 Å,  $\lambda$  4916 Å and  $\lambda$  3821 Å all exert a slight inhibitory action, whereas all other lines used, whether in the visible or ultra-violet spectrum, exert a decided stimulation, in some cases exerting stimulation identical with that caused by the same lines on the dextrinogenic action and in other cases being the direct opposite.

TABLE VIII

THE SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE SACCHAROGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Wave-length	Irradiated	Control	% Diff.	Average effect
6152 Å	15.5	16.0	3.2	-5.15
	14.0	15.0	7.1	
5819 Å	10.0	9.0	10.0	9.0
	12.5	11.5	8.0	
5461 Å	37.0	29.0	21.6	21.7
	25.0	19.0	26.9	
	20.0	17.0	15.0	
4916 Å	24.0	24.0	0	1.0
	25.0	24.5	2.0	
4359 Å	16.0	12.0	25.0	19.1
	18.0	14.0	22.2	
	35.0	31.5	10.0	
4078 Å	24.0	24.0	0	2.7
	21.0	20.0	5.0	
	15.0	14.5	3.3	
3821 Å	27.0	27.0	0	-1.5
	15.0	15.5	3.0	
3663 Å	17.0	16.0	6.0	11.2
	18.0	15.0	16.5	
3132 Å	36.0	28.0	22.2	21.7
	38.0	30.0	21.2	
3022 Å	18.0	16.0	11.1	26.6
	26.0	17.0	34.5	
	28.0	18.0	35.7	
	16.0	12.0	25.0	
2967 Å	19.0	14.0	26.3	25.0
	21.0	16.0	23.8	
2804 Å	17.0	17.0	0	0
2700 Å	19.0	19.5	0	.5

These results are a little difficult to explain, owing to the fact that illumination from the full mercury spectrum caused decided inhibition in saccharogenic activity. These results, however, may have some bearing on those of H. J. Fuller, who reports that the activity of catalase and plant diastase is increased to a considerable extent in tissues exposed to otherwise inhibitory wave-lengths, and found no diminution of enzyme activity. A possible explanation is offered later under the heading of "pace setter".

In comparing these two sets of results there is evidence that, while the lines of the mercury arc spectrum which exert a measurable effect on the dextrinogenic activity of the malt diastase, cause inhibition, the effect of the monochromatic lines, except those of the red end of the visible spectrum, on the saccharogenic activity is stimulatory.

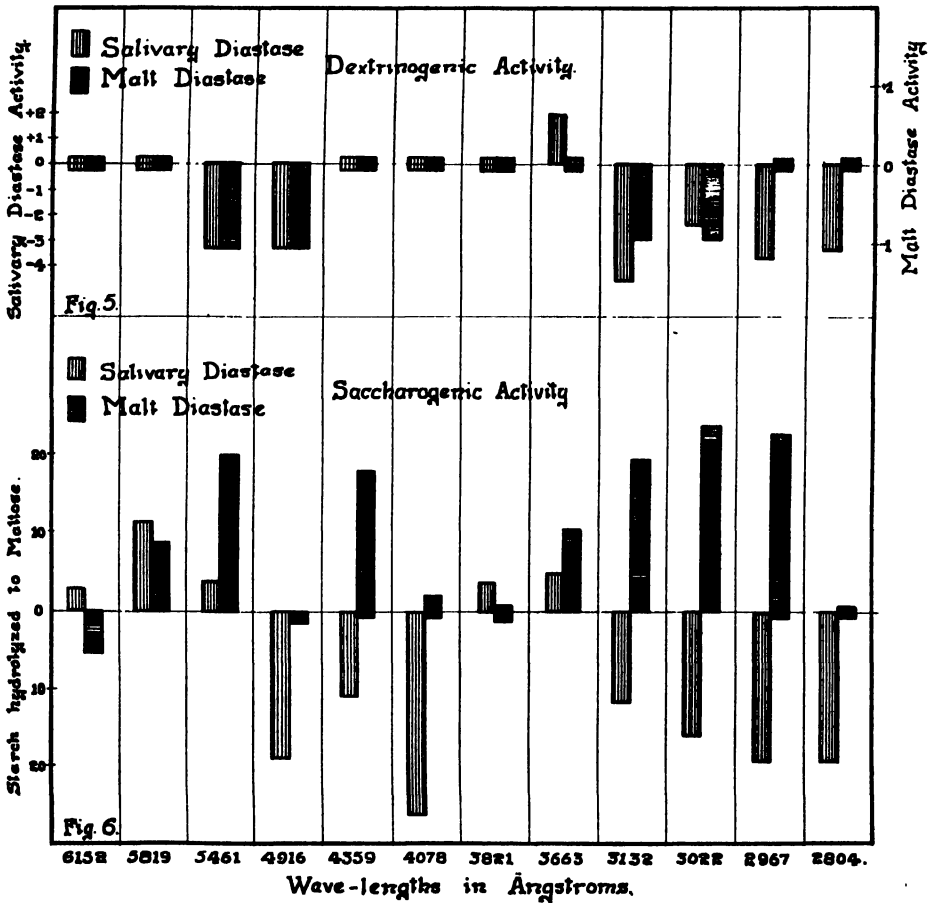


FIG. 5. A graph to compare the specific effects of various light wave-lengths on the dextrinogenic activity of salivary and of malt diastase.

FIG. 6. A graph to compare the specific effects of various light wave-lengths on the saccharogenic activity of salivary and of malt diastase.

*Comparison of the Effects of Irradiation on the Dextrinogenic Activity of the Two Diastases (Fig. 5)*

In these experiments the salivary diastase is most affected by the irradiation used, there being two portions of the spectrum, that around the green region of the visible spectrum, and that in the far ultra-violet region, which cause distinct inhibition of action. One line only,  $\lambda$  3663 Å, exerts a stimulating effect. The malt diastase is at no time stimulated, and although inhibited by the same wave-lengths of the green region as is salivary diastase, inhibition is confined elsewhere to two lines,  $\lambda$  3122 Å and  $\lambda$  3022 Å; the far ultra-violet apparently having no appreciable effect in this case.

*Comparison of the Effect of Irradiation on the Saccharogenic Activity of the Two Diastases (Fig. 6)*

In the case of the salivary diastase the red-yellow and the near ultra-violet portions of the spectrum exert stimulation, but the region of the green-violet lines and the far ultra-violet exert decided inhibition of enzyme action, the visible lines giving an even greater effect than the ultra-violet lines. On the other hand, with the exception of  $\lambda$  6152 Å,  $\lambda$  4916 Å and  $\lambda$  3821 Å, which exert an almost negligible negative effect, the malt diastase activity is greatly stimulated, not only by the lines of the visible spectrum but also by the ultra-violet lines of intermediate wave-length. The only wave-lengths which have like effects on the malt and salivary saccharogenic activity are the orange, yellow and near ultra-violet.

*Comparison of the General Effect of Irradiation on the Two Diastases*

It would seem, in the experiments here reported, that the effect of irradiation is greater in the case of the saccharogenic activity of both enzymes, and that the dextrinogenic activity is affected to a lesser extent.

It is also of interest to note that salivary diastase, on the whole, is affected to a greater extent than malt diastase, and that the inhibitory effect predominates. In malt diastase, the dextrinogenic activity is inhibited by only four lines, and the saccharogenic activity is generally stimulated.

*The Diastatic "Pace Setter"*

The production of erythrodextrin is dependent upon the activity of the dextrinogenic enzyme phase, while that of maltose is dependent on the two phases, dextrinogenic and saccharogenic. However, since the slowest action in any series is the "pace setter", and since the effects of ultra-violet light on the dextrinogenic action is similar in the two cases, these experiments indicate that the dextrinogenic activity is the "pacesetter" for salivary diastase while the saccharogenic activity is the "pace setter" for malt diastase. This result seems to agree with the conclusions of Sherman, Schlesinger and Kendall (10, 14), that the liquefying action is predominant in salivary diastase, while the saccharifying action is predominant in malt diastase. The parallelism between the measured effects on the dextrinogenic phases of hydrolyses for salivary and malt diastase indicates that the dextrinogenic phases are similar in both

enzymes. It is possible that the effect on the saccharogenic enzyme phases may be the same in each also, but in the case of the salivary diastase the effect is masked by the controlling dextrinogenic phenomena. The saccharogenic effect on malt diastase is controlled by the dextrinogenic phase only when the intensity of irradiation is sufficiently great to constitute the latter as "pace setter", as in the case of full irradiation.

#### *Evidence for Two Enzymes*

The differences in the effects of monochromatic light upon the saccharogenic activity of malt diastase as compared with salivary diastase, in contrast with the similarities of the dextrinogenic effects together with the inversion of the effect of monochromatic light as compared with that of full irradiation on the saccharogenic activity of malt diastase, indicate that the two phases of starch digestion are each dependent upon a separate enzyme or that the enzyme changes its light relations. The results are more readily explained by postulating two enzymes, one dextrinogenic, and the other saccharogenic. Under ordinary conditions, as in malt diastase, the saccharogenic may be the "pace setter". Light may retard the activity of the dextrinogenic to such an extent that it becomes the "pace setter". This evidence is in accord with the conclusions of Sherman, Schlesinger, Kendall and others on the nature of the enzymes involved in the hydrolysis of starch (10, 14).

#### *A Comparison of the Effect of Monochromatic Light on the Growth of Paramecium, and on the Diastatic Activity of Saliva (Fig. 7)*

The very close parallelism is illustrated by Fig. 7. Of the wave-lengths tested, two only show effects differing in direction and these ( $\lambda$  5461 Å and  $\lambda$  3821 Å) are in transition regions, between regions of stimulatory and inhibi-

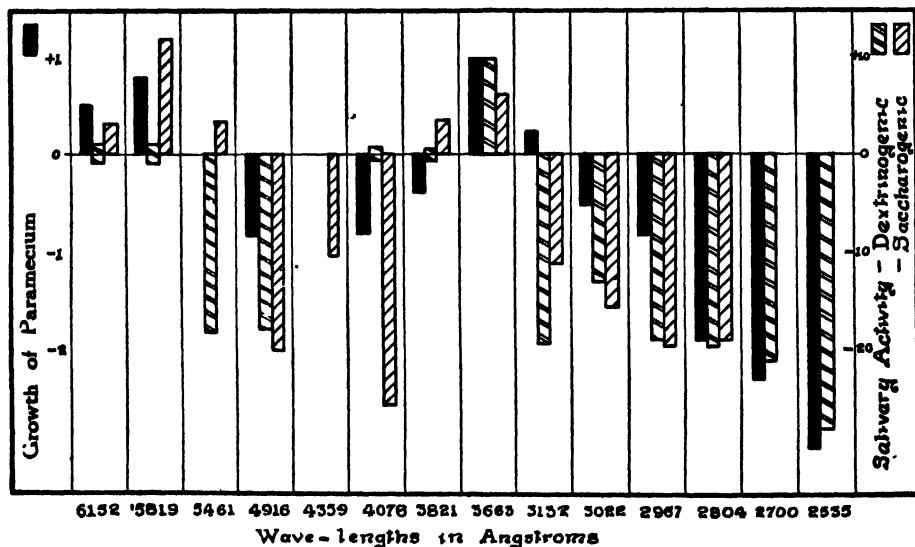


FIG. 7. A graph to compare the effects of various light wave-lengths upon the growth of paramecium and the activity of salivary diastase, dextrinogenic and saccharogenic.

tory effects. For other wave-lengths not only is stimulation or retardation of growth associated with stimulation or retardation of the enzyme activity respectively, but, in most instances, the magnitudes of the two effects are relatively in the same order. This parallelism is even more remarkable since the enzyme is that of man while the rate of growth is that of a unicellular organism. At present data are not available for the diastase of paramecium. The parallelism suggests that the growth of certain organisms may be limited by the food made available by enzymes such as diastase.

*A Comparison of the Effect of Monochromatic Light on the Sporulation of Colletotrichum and on the Saccharogenic Activity of Malt Diastase*

Of the several phenomena studied by the authors (6, 7, 8, 9), including the effect on growth of several organisms (6, 7, 9), the effect on plasmolysis (8), the effect on sporulation (7) and on enzyme activity, only two exhibit

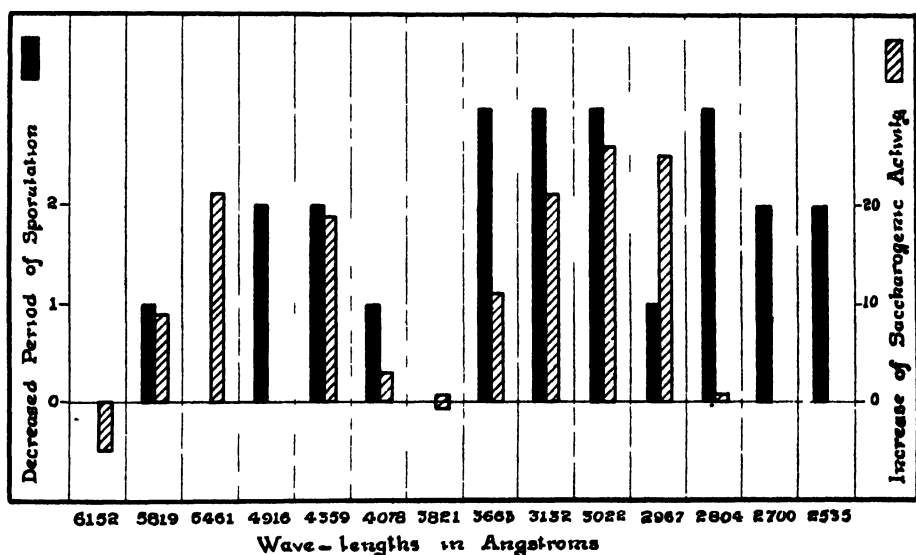


FIG. 8. A graph to compare the effects of various light wave-lengths upon the sporulation of *Colletotrichum* (Tomato rot) and the saccharogenic activity of malt diastase.

a consistent positive response to the several wave-lengths of monochromatic light, namely, sporulation in *Colletotrichum* and the saccharogenic activity of malt diastase. The parallelism is shown in Fig. 8. Spores frequently store abundant sugar and this is probably true of *Colletotrichum*. The parallelism suggests that the formation of spores may be limited by the production of maltose as regulated by saccharogenic enzymes.

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# AN INVESTIGATION OF THE SURFACE TENSION OF LIQUIDS NEAR THE CRITICAL TEMPERATURE<sup>1</sup>

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## Abstract

The surface tension-temperature relations of methyl ether and propylene have been investigated by the capillary rise method over a range of temperatures approaching more closely to the critical temperature than has hitherto been done. From the data obtained, it has been concluded that the molecular surface energy does not become equal to zero when the meniscus is no longer visible, since the surface tension-temperature curves obtained apparently did not become asymptotic to the temperature axis at the critical temperature, the latter being considered as the temperature at which the meniscus is no longer discernible by the wave-lengths of visible light. The angle of interception of the surface tension-temperature curve with the temperature axis has been interpreted as indicating a discontinuity in properties at the critical point.

The data obtained have also been applied to the examination of various relations involving surface tension. The Katayama equation has been shown to be considerably more accurate than that of Ramsay and Shields. Sugden's equation, has been found valid over the ranges of temperature investigated. The Macleod relation has also been examined, and an increase in the Macleod constant with increase in temperature shown to occur in the case of propylene, while no marked or progressive increase was noticed in the case of methyl ether. Calculation of the parachors served to emphasize the difference in behavior of the two substances in this respect. By a consideration of available data on benzene, chlorobenzene, and carbon tetrachloride, together with those obtained in this investigation, the increase of parachor in the case of those substances having unsaturated linkages in their molecular structure has been ascribed to increased electronic interaction with increased temperature. This is analogous to an increase in unsaturation.

An attempt was made to adapt the ring method to the investigation of surface tension in the critical region, by measuring the force necessary to effect removal of the ring from the surface of the liquid by means of a calibrated quartz spiral. Although the method was unsatisfactory for the problem at hand, the feasibility of the method has been demonstrated. From the data obtained it has been possible to verify the zero angle of contact between methyl ether and glass.

## Introduction

The accurate determination of the surface tension of liquids has received the attention of numerous workers. The rise of liquids in capillary tubes was first studied by Leonardo da Vinci, in the seventeenth century, but it was not until von Segner (17) introduced the conception of surface tension that the subject became of fundamental interest and importance. The first correct theory of the rise of liquids in capillary tubes was put forward by Leslie (8) in 1802, but the mathematical treatment was not expounded until Young (26), Laplace (7), and others gave it their consideration. Since that time, the theory of surface tension has received considerable modification and development. Paralleling the theoretical development, the experimental technique for determining the surface tension of a liquid has also advanced to a remarkable extent. New methods have been devised,

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and older methods modified; according to Ferguson (5) some 20 methods have been investigated, of which the capillary rise method and the ring method are probably most commonly used at the present time.

The temperature coefficient of surface tension has also received considerable attention. In general, it is not large (about 0.5% per degree), and is negative. Over short temperature ranges, the surface tension-temperature relation is practically linear, but observations over extended ranges of temperature (13) show that the surface tension-temperature graph exhibits a decided curvature. Furthermore, a rapid diminution in the slope of the curve near the critical temperature was observed.

A careful review of the published data reveals that, although the temperature coefficients of surface tension for numerous liquids are well established for considerable temperature ranges, surprisingly few investigations have been extended to the critical point. In all, probably not more than six liquids have been studied at temperatures approaching the critical, and even for these the investigations have not included temperatures closer than approximately 5°C. to the critical temperature. Graphical representation of the published data for these substances indicates that the surface tension diminishes to zero value at the critical point.

Recent investigations in this laboratory suggested the possibility, however, that partial orientation of the molecules at the liquid-vapor interface is maintained even after the critical temperature is attained. Should this be the case, the surface tension-temperature curve might be expected to extrapolate to zero value of the surface tension at some temperature above the critical. Although purely speculative in its origin, this problem demanded experimental investigation, since the data available were not sufficient to provide the solution. Consequently, it was decided to determine the surface tension of some liquids over as large a temperature range as possible, in an endeavor to establish the surface tension-temperature relation closer to the critical temperature than had previously been done.

## Experimental

Of the numerous methods for the determination of surface tension, the ring and the capillary rise methods were deemed most readily applicable in the critical region. The former proved to be somewhat cumbersome, however, and was rejected in favor of the capillary rise method. A brief description of the ring method will be given, since the manner in which it was applied to the present problem, and the results obtained thereby, might prove of considerable interest.

### (a) *The Ring Method*

This method, initiated by Sondhaus (18), and developed to some extent by Timberg (23) and Weinberg (24), did not become generally applicable to the determination of surface tension until Du Noüy (3, 4) devised the tensiometer which bears his name. The principle employed by Du Noüy did not

seem applicable, however, in a system which must be closed, under a pressure of some 50 atmospheres, at a temperature of approximately 125°C. Consequently, a quartz spiral replaced the torsion balance for measuring the force required to effect removal of the ring from the surface of the liquid.

A platinum ring, of approximately 1 cm. circumference, was suspended from a machine-wound, calibrated quartz spiral, the sensitivity of which was 0.00194 gm. per mm. extension. For details concerning the method of winding such spirals, the reader is referred to a paper by Tapp (22), who very kindly furnished the spiral used in this investigation.

The necessity for obtaining a uniform upward motion of the ring through the surface of the liquid presented a problem of very great difficulty; indeed, the ring method was finally abandoned for this reason. Many devices, which promised successful operation, had to be abandoned in actual practice. The apparatus finally employed was not all that was desired from the standpoint of manipulative ease, but did indicate the feasibility of the method, and gave promise of the development of a satisfactory technique for the determination of the surface tension of a liquid, near the critical temperature, by the ring method.

The spiral and attached ring were suspended by a silk thread from a glass sheath, which enclosed a small iron nail. The thread was of a length calculated to satisfy the required elevation of the ring, as determined by the dimensions of the complete assembly. At each end of the sheath, symmetrically about it, were sealed three fine glass rods, at right angles to the axis of the nail. The lengths of these rods were such that they did not quite touch the inner wall of the glass bomb which was to contain the mechanism. In this manner, the sheath around the nail was held centrally in the bomb, and made contact with the wall at only four points at any time.

The bomb which contained the spiral assembly was of Pyrex tubing, with an internal diameter of 1.5 cm., and a wall thickness of 4 mm. After sealing off one end of the tube, indentations were made in the wall at an appropriate distance from the bottom, or sealed end, these indentations being diametrically opposite. A short piece of tubing, inserted into the bomb and allowed to rest on the indentations, served to support the spiral assembly while the apparatus was not in use, since, upon lowering the assembly, the short projecting rods on the sheath came to rest on the upper edge of this short tube. At a calculated distance above the sheath, the bomb was drawn down, and a short piece of capillary tubing sealed to it, through which the liquid could be introduced.

Methyl ether was introduced by low temperature distillation, until the liquid occupied  $\frac{2}{3}$  of the total bomb space, at  $-78.5^{\circ}\text{C}$ . This ratio of liquid to total bomb space was maintained to minimize the motion of the meniscus near the critical temperature. The bomb was then sealed off, and immersed



FIG. 1. Diagram of the apparatus for the ring method.

in a bath of glycoline oil. The bottom of the bomb rested on a cork support; to the top was fixed a collar supported on one end of a long brass rod, which extended some distance from the bath. At the other end of this rod was fixed a double system of racks and pinions, which enabled the operator, at a distance from the bath, to move the top of the bomb in any direction. This enabled the operator to keep the ring in the centre of the liquid surface, a necessary feature, since there was a slight tendency for the ring to draw towards the wall of the bomb at the instant of release.

To further decrease the tendency for the glass sheath surrounding the nail to bear lightly against the wall of the bomb, and thus impair the production of an even force on the ring, a very rapid, but very slight, vibration was imparted to the upper portion of the bomb. This was done by permitting the core of a small solenoid, through which an alternating current was passing, to bear very lightly against the bomb. The amount of vibration could be regulated and was never permitted to increase to an extent which would cause small ripples on the surface of the liquid in the lower portion of the bomb.

The spiral was raised by means of a small solenoid placed around the bomb. In this way, the force tending to draw the nail over to the wall in one direction was never greatly in excess of that tending to draw it in another, a factor which aided in the attainment of a steady pull on the ring. This solenoid could be raised and lowered by the operator at some distance from the bath.

The temperature of the bath was controlled manually, and ascertained on a standard thermometer, with an error not exceeding  $0.03^{\circ}\text{C}$ . The extension of the spiral was determined with a cathetometer reading to 0.02 mm. In observing this extension, it was necessary to follow the upper extremity of the spiral, as it was being raised, with the cathetometer, the reading at the instant that the ring broke from the surface being noted. The release of the ring from the surface was accompanied by a slight, but detectable, jerk in the upward motion of the spiral. In any case where this was not noticed, the reading was not recorded. At the higher temperatures, the release was not as noticeable as at the lower temperatures, but with care could be fairly readily detected. The reading at the point at which the meniscus stood was taken in each case, and the difference between this reading and that of the upper extremity of the spiral, minus the normal length of the spiral with ring attached, gave the extension produced in the spiral due to the force necessary to release the ring from the surface of the liquid. Since the spiral was calibrated, the only factor remaining unknown was the circumference of the ring. This was determined in a manner to be indicated later, after which the surface tension of methyl ether was readily calculable.

#### *(b) The Capillary Rise Method*

The capillary rise method was finally adopted for this investigation for two main reasons. A high degree of accuracy was of fundamental importance, since a small surface tension prevails in the region it was desired to investi-

gate. The capillary rise method answers this requirement, being probably the most accurate method yet devised. Furthermore, it soon became evident that the capillary rise method should be more readily applicable under the experimental conditions encountered than the ring method first attempted.

While it is not the intention to review in detail the very extensive literature dealing with the determination of surface tension by the rise in a capillary tube, some of the important considerations indicated by the work of others might be of interest. Richards and his coworkers (14, 15, 16) have demonstrated the necessity for scrupulous cleanliness, uniformity and non-ellipticity of the bore of the capillary tube, smoothness of its internal surface, and good optical properties of the capillary walls. They have also indicated the advantages and disadvantages of various arrangements of the smaller and larger tubes of the capillarimeter. Predescu (12) suggested a modification in the construction and manipulation of the apparatus which reduces the error due to variations in the bore of the capillary tube. Sugden (19) described a modified capillarimeter designed to obviate difficulties arising from the absence of a plane surface of reference.

In preliminary work, the smaller capillary was sealed at both its ends externally into the wall of a larger capillary tube, a design which is desirable from the standpoint of accuracy. The technical difficulties encountered in constructing such a capillarimeter proved to be very great. Although it was possible, after a number of attempts, to obtain sufficient mechanical strength to withstand the pressures imposed, it was not found possible to control the temperature of the apparatus sufficiently well with the facilities available. While fluctuations in the temperature of the bath as a whole, as recorded thermometrically, could be reduced to the desired extent, slight regional fluctuations could not be eliminated. Distillation of the liquid from one portion to another of the smaller capillary as a result of these regional fluctuations in temperature necessitated the abandonment of this type of capillarimeter.

The second type of apparatus used proved successful. In this type, the small capillary was contained inside a heavy-walled Pyrex glass bomb. Since the external diameter of the capillary was approximately 1 mm. as compared with an internal diameter of 15 mm. of the larger tube, the surface of the liquid was practically plane, especially at the higher temperatures. Consequently, the degree of accuracy was estimated to be not greatly inferior to that attainable with the first type of capillarimeter.

Calculation showed the necessity for a capillary of approximately 0.1 mm. internal diameter, to permit of sufficient accuracy. One satisfying this requirement, and also the other specifications mentioned, was selected.

After cleaning successively with chromic acid, distilled water, alcohol and ether, and drying carefully, the capillary was inserted into a short piece of capillary tubing of the proper bore to hold it rigidly. This supporting capillary was chosen of such an external diameter that it fitted tightly into the larger bomb tubing.

The bomb was of Pyrex glass, with a wall thickness of 4 mm., and an internal diameter of 15 mm. It was selected with special reference to its optical properties. After thorough cleaning and drying, one end was sealed off, and two indentations made in the wall at diametrically opposite points. The capillary tube, mounted as previously described, was then inserted, and forced down until the supporting short piece of capillary came to rest on the indentations in the wall of the bomb. Care was taken to ensure a vertical position of the capillary. The bomb was then sealed to a low temperature fractionating unit, and the desired liquid introduced by distillation.

Methyl ether was prepared and purified as described by Winkler and Maass (25). Propylene was prepared and purified to constant vapor pressure as outlined by Coffin and Maass (2) and Maass and Wright (10). The pure gas (either methyl ether or propylene) was condensed into the bomb by surrounding it with a freezing mixture of solid carbon dioxide and acetone, after which it was sealed off.

The temperature was controlled by immersing the bomb in a bath of electrically heated glycoline oil. A thermoregulator was provided to maintain the temperature constant to within  $0.2^{\circ}\text{C}.$  of the desired temperature. Closer regulation was effected by manual control of the rheostats connected in series with the heating element, as will be described later. The temperature was ascertained on a standardized thermometer, with an error not exceeding  $0.03^{\circ}\text{C}.$  Owing to the possibility of the bomb shattering at the higher temperatures, the operator was protected by a housing which enclosed the apparatus, a non-shatterable glass window being provided through which the observations could be made. The thermometer was read by means of a telescope.

The cathetometer used in the investigation was accurate to 0.02 mm. over the range required. It was fitted with a microscope objective (16 mm.), which enabled the meniscus in the fine capillary to be seen with ease, even at temperatures very close to the critical.

Preliminary work showed the necessity of having a very slight temperature gradient from the top of the bath to the bottom. This was attained by adjusting the positions of the heating elements, and regulating the rate of stirring. With such a temperature gradient, never permitted to exceed  $0.1^{\circ}\text{C}.$ , the distillation of the liquid in an upward direction in the fine capillary was prevented.

Furthermore, it soon became evident that, having set the thermoregulator for a given temperature, several hours elapsed before equilibrium was established. Owing to the slow attainment of equilibrium, the bomb was usually permitted to remain overnight at the desired temperature, regulated within  $0.2^{\circ}\text{C}.$  Successive readings of the capillary rise were then taken every half hour, the temperature being held constant, by manual control, to within  $0.03^{\circ}\text{C}.$  Equilibrium was considered to be established when three successive readings, at half hour intervals, did not differ by an amount exceeding 0.04 mm.

Having made the required measurements of the capillary rise for methyl ether and propylene, the capillary tube was removed and calibrated over its entire length. This was done by introducing a short thread of mercury, and measuring its length at successive positions in the tube by means of a horizontal cathetometer. This mode of calibration has been shown by Richards and Carver (14) to be sufficiently accurate, the presence of a film of air between the mercury and glass not introducing an appreciable error. The length of the mercury thread at successive positions was ascertained with an error not exceeding 0.1%.

Since a balance sufficiently sensitive to weigh the quantity of mercury constituting the thread was not available, and since relative values of the surface tension were adequate, the diameter of the capillary was measured at one point with a microscope fitted with a micrometer eyepiece. The error did not exceed 1%, and as will be evident later, was considerably less than this amount. No difference in diameter was detectable by making measurements at right angles, indicating the absence of appreciable ellipticity in the bore.

From a knowledge of the relative values of the radius at various points in the capillary, as provided by the mercury thread calibration, the radius itself, corresponding to any position in the tube could be calculated from the value determined microscopically. From a calibration curve, the appropriate value for any experimentally determined capillary rise could be readily ascertained.

The densities of the liquid and vapor phases for methyl ether, necessary for the calculation of the surface tension, were taken from the data of Cardoso and Cappola (1). Data for the densities of liquid and gaseous propylene, over the temperature range employed in the present investigation, are not available in the literature. Consequently, these were determined; the method adopted and the results obtained will be discussed in a future paper dealing with the densities in the liquid and gaseous phases in the critical region.

### Results and Discussion

The results obtained by the capillary rise method will be tabulated and discussed before those obtained by the ring method, since the former served as a basis for the calibration of the ring used in the latter method.

#### CAPILLARY RISE METHOD

The surface tension was calculated for each liquid, at a given temperature, from the well-known formula relating the surface tension to the rise of a liquid in a capillary tube, where the angle of contact is zero, namely,  $T = \frac{rgh(D-d)}{2}$ , where  $T$  is the surface tension of the liquid in dyne/cm.;  $r$  is the radius of the capillary in cm.;  $g$  is the gravitational constant;  $h$  is the rise in cm., of the liquid in the capillary tube; and  $D-d$  is the difference in density of liquid and vapor, at the temperature employed. The degree of accuracy sought did not necessitate a correction for the volume effect of the meniscus.

The results obtained for the surface tension of methyl ether are shown in Table I, and graphically represented in Fig. 2, while those for the surface tension of propylene are set forth in Table II, and graphically represented in Fig. 3. The experimental data for propylene are considered to be somewhat more accurate than those for methyl ether, owing to the greater facility with which the temperature could be controlled in the case of the former.

TABLE I

THE RELATION BETWEEN THE SURFACE TENSION OF METHYL ETHER AND TEMPERATURE

Temp., °C.	102.50	109.50	115.00	122.00	124.00	125.00
S.T., dyne/cm.	1.86	1.25	0.78	0.24	0.11	0.05

Although relative values of the surface tension were adequate for the solution of the problem originally conceived, the data obtained probably deviate less than 5.0% from the true values. This deviation, should it exist, is to be accounted for by the error arising in the microscopic ascertainment of the radius of the capillary at the one point, to which the radii at other points are referred. This method however has been adopted by Sugden (20) in much of his work on the surface tension of liquids. In any case, the relative values are considerably more accurate than the limit quoted above, and hence the conclusions drawn should be quite valid.

The variation of the surface tension with temperature at the critical temperature was the main objective. Whether or not this objective has been attained must be discussed from the actual observations which have been made. The question, of which the answer was sought, may be stated thus: is the tangent to the surface tension-temperature curve definitely at an angle of 90 degrees to the surface tension axis, at the critical temperature? The degree of certainty with which this question may be answered depends on the proximity to the critical temperature of the last point observed on the temperature scale, and the magnitude of its ordinate, these to be correlated to the experimental error involved. The last observation which could be made with certainty was approximately 1.5°C. below the critical temperature,

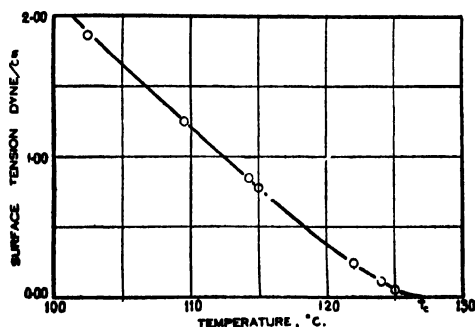


FIG. 2. The surface tension-temperature relation for methyl ether.

with an ordinate value that could be ascertained with an error not exceeding 5%. Taking this point with those points preceding, and extrapolating on a semi-linear basis, there is a gradient which is not zero at the critical temperature.

On the other hand, it is conceivable that determinations made above the last temperature attained might fit into an asymptotic surface tension-temperature curve at the critical tem-

TABLE II

THE RELATION BETWEEN THE SURFACE TENSION OF PROPYLENE AND TEMPERATURE

Temp., °C.	62.10	73.20	77.80	83.60	88.20	89.60	90.50
S.T., dyne/cm.	2.40	1.34	0.94	0.50	0.17	0.09	0.05

perature. Assuming this to be the case, a very marked inflexion in the surface tension-temperature curve would have to be admitted, this inflexion occurring quite close to the critical point. This is not inconceivable, since there are marked variations in density, with temperature, in this region; nevertheless, the results obtained would indicate the possible existence of pseudo-negative surface tension above the critical temperature. A negative surface tension is quite as impossible as a negative temperature on the absolute scale; the word - "pseudo-negative" is used, therefore, to indicate a measurement which would be dependent upon a *visible* position of the meniscus above the critical point.

The meniscus is usually defined as a visible line of demarcation between the liquid and vapor phases; this implies a thickness such that its presence may be ascertained through the agency of visible light. This definition may well be extended, however, to include dimensions not detectable by the wave-lengths of ordinary, or visible, light. In so doing, the existence of surface energy is not denied; if a difference in density between two media persisted, the surface energy connected with the passage from that of the greater to that of the lesser density would be reflected in a definite angle of interception of the surface tension-temperature curve with the zero surface tension axis, even if the meniscus disappears in so far as optical detection is concerned.

From a consideration of the data obtained, the authors are of the opinion that a definite difference between the density of the liquid and that of the

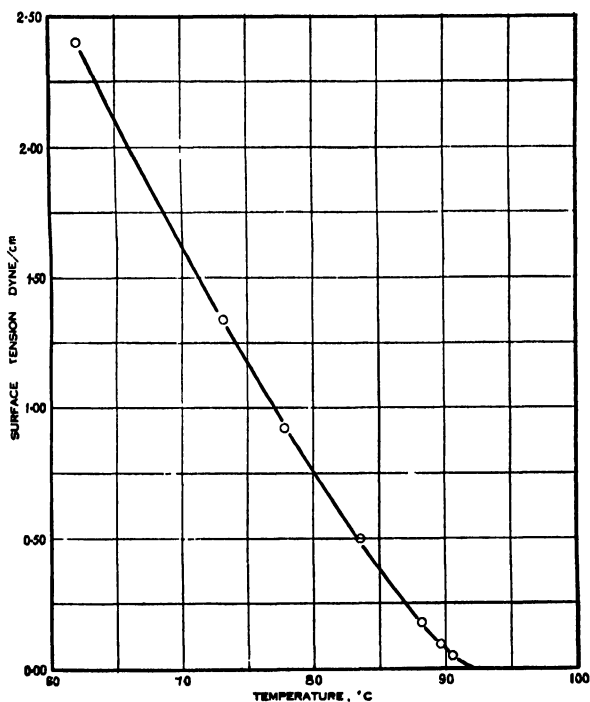


FIG. 3. The surface tension-temperature relation for propylene.



vapor persists above the so-called critical temperature. This conclusion has been confirmed in an investigation of the densities in this region, the results of which will be communicated in a future paper.

Having applied the data to the solution of the original problem, as above, additional information of considerable value can be obtained from a few fundamental considerations. As pointed out previously, the available data for the surface tension of liquids near the critical point are very limited. Since the relative values of the surface tension obtained in this investigation are quite accurate, they can be profitably applied to the examination of various relations involving surface tension. The actual values of the constants, etc., which can be evaluated, are possibly slightly in error, although, as will become evident later, the calculation of these constants points to a high degree of accuracy, even in so far as the actual values of the surface tension are concerned.

(a) *Molecular Surface Energies of Methyl Ether and Propylene*

The molecular surface energies for methyl ether and propylene were calculated at each temperature, together with the Ramsay and Shields constant over the corresponding temperature intervals. The results obtained are shown in Table III. It is evident, from the rapid diminution in the value of the Ramsay and Shields constant for methyl ether, that their equation is not applicable at temperatures approaching the critical. At first glance, the value of  $k$  would appear to be considerably too low. Maass and Boomer (9) however report a decrease in the value of  $k$  for methyl ether from 2.12 at  $-42.0^{\circ}\text{C.}$  to 1.76 at  $-10.6^{\circ}\text{C.}$ , which brings the values obtained in the present investigation in line with those at the lower temperatures.

TABLE III

THE MOLECULAR SURFACE ENERGIES AND THE RAMSAY AND SHIELDS AND KATAYAMA CONSTANTS FOR METHYL ETHER AND PROPYLENE

Temp., °C.	$S(M/D)^{\frac{1}{2}}$	$k$ (R & S)	$S(M/D-d)^{\frac{1}{2}}$	$k_1$ Katayama	Temp., °C.	$S(M/D)^{\frac{1}{2}}$	$k$ (R & S)	$S(M/D-d)^{\frac{1}{2}}$	$k_1$ Katayama
<i>Methyl ether</i>					<i>Propylene</i>				
102.50	38.64	1.67	44.15	1.72	62.10	52.0	1.97	58.8	2.09
109.50	26.93	1.73	32.07	1.87	73.20	30.2	1.80	35.6	1.93
115.00	17.40	1.66	21.76	1.92	77.80	21.9	1.70	26.7	1.84
122.00	5.77	1.57	8.28	2.08	83.60	12.1	1.65	16.0	1.95
124.00	2.63	1.36	4.11	1.88	88.20	4.5	1.43	7.0	1.89
125.00	1.28		2.23		89.60	2.5	1.32	4.3	1.92
					90.50	1.3		2.6	

In the case of propylene also the rapid diminution in the value of  $k$  near the critical temperature is evident, and is quite in line with the results obtained by Maass and Wright (10) at lower temperatures.

The validity of the equation developed by Katayama (6) has been subjected to a critical examination by means of the results obtained in this investigation.

His equation is of the form,  $S(M/D-d)^{\frac{1}{3}} = k_1(T_c - T)$ , where  $S$  is the surface tension of the liquid;  $M$  is the molecular weight of the substance;  $D-d$  is the difference in densities of liquid and vapor at temperature  $T$ ; and  $k_1$  is Katayama's constant.

The values for the molecular surface energies calculated from this equation, also the values of  $k_1$ , are shown in Table III for methyl ether and propylene. It is of interest to note that the value of  $k_1$  remains quite constant, even at temperatures very close to the critical, indicating the advantages of the Katayama equation over the classical equation of Ramsay and Shields.

(b) *The Relation Between Temperature and the Surface Tensions of Methyl Ether and Propylene*

In 1894, van der Waals suggested the following formula to express the variation of surface tension with temperature:

$$S = K_1 T_c V_c^{-\frac{1}{3}} (1 - T_r)^b = K_2 T_c^{-\frac{1}{3}} P_c (1 - T_r)^b.$$

Here  $T_c$ ,  $V_c$ , and  $P_c$  are the critical temperature, volume, and pressure respectively, and  $T_r$  the reduced temperature.  $K_1$  and  $K_2$  and  $b$  are universal constants. Sugden (20) has obtained this equation in the form,  $S = S_0(1 - T_r)^{\frac{2}{3}}$ , where  $S_0$  represents the surface tension of the supercooled liquid at the absolute zero. If the equation accurately represents the variation of the surface tension with temperature, a plot of  $S^{\frac{3}{2}}$  against temperature should give a straight line. Sugden has found that the equation holds accurately, and a linear relation is obtained, provided the liquid is not associated. With associated liquids, the graph takes on a decided curvature.

In Fig. 4 are plotted values of  $S^{\frac{3}{2}}$  for methyl ether and propylene, for values of  $S$  ascertained from Figs. 1 and 2, at 5°C. intervals of temperature. It is quite evident that the equation is valid for the two liquids under investigation, from which it is to be concluded that methyl ether and propylene are not associated at the temperatures employed.

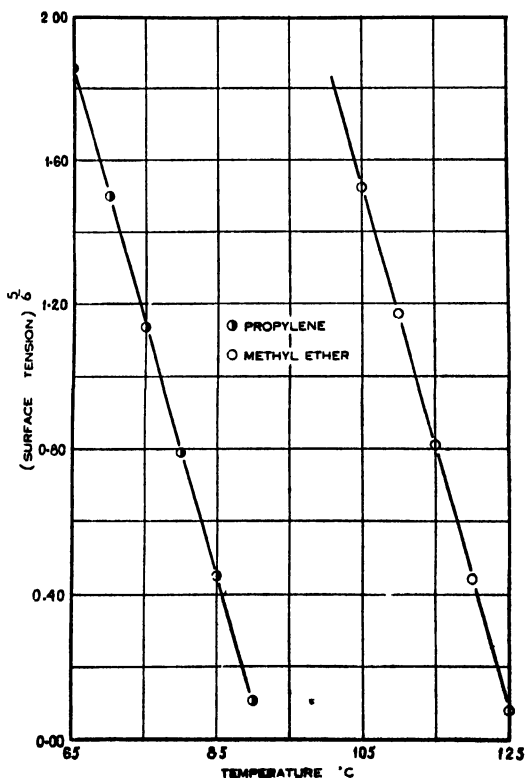


FIG. 4. Relation between temperature and  $(S.T.)^{\frac{1}{2}}$ .

(c) *The Relation Between the Surface Tensions and Densities of Methyl Ether and Propylene*

A relation between surface tension and density has been obtained by Macleod (11). His equation is of the form  $S = C(D-d)^4$ , where  $D$  and  $d$  are the densities of the liquid and vapor, and  $S$  is the surface tension. The constant,  $C$ , has been found invariable with temperature up to the neighborhood of the critical temperature.

The values of  $C^{\frac{1}{4}}$  at various temperatures have been calculated for methyl ether and propylene, and are shown in Table IV. For methyl ether, there seems to be no definite and progressive increase in the value of  $C^{\frac{1}{4}}$ , but a marked and progressive increase is noticeable in the case of propylene. An increase in the value of this constant has been noticed near the critical point in the cases of benzene and chlorobenzene, but not in the case of carbon tetrachloride. The tendency for the value of  $C^{\frac{1}{4}}$  to increase near the critical temperature has been attributed to errors in the surface tension and density measurements in this region. An alternative explanation will be postulated in connection with the parachors of these substances.

(d) *The Parachors of Methyl Ether and Propylene*

The parachors of methyl ether and propylene at various temperatures are shown in Table IV. Although the parachor of methyl ether does not deviate largely or consistently from the theoretical value of 132.2, that of propylene shows a marked increase over the theoretical value of 140.2, as the critical temperature is approached.

TABLE IV  
THE MACLEOD CONSTANT AND THE PARACHOR FOR METHYL ETHER AND PROPYLENE

Temp., °C.	$C^{\frac{1}{4}}$	$MC^{\frac{1}{4}}$ *	Temp., °C.	$C^{\frac{1}{4}}$	$MC^{\frac{1}{4}}$ **
<i>Methyl ether</i>			<i>Propylene</i>		
102.50	2.94	136.0	62.10	3.60	151.2†
109.50	2.99	137.4	73.20	3.51	147.4
115.00	3.02	138.9	77.80	3.53	148.3
122.00	3.08	141.8	83.60	3.64	152.9
124.00	3.03	139.3	88.20	3.93	165.0
125.00	2.96	136.2	89.60	4.29	180.2
			90.50	4.68	196.5

\* $MC^{\frac{1}{4}}$  (theoretical) = 132.2.    \*\* $MC^{\frac{1}{4}}$  (theoretical) = 140.2.    †This value is probably too high, owing to the necessity of extrapolating the density data for the calculation of the surface tension.

To account for the large and progressive deviation of the propylene parachor from the theoretical value on the basis of experimental error alone does not seem justified. Like the increase in the Macleod constants for benzene and chlorobenzene, which have been accounted for on this basis by Sugden, the increase in the parachors of these substances is essentially explained in the same way, if no alternative explanation can be offered. It is significant,

however, that for benzene and chlorobenzene, as for propylene, the progressive increase in parachor becomes noticeable at temperatures as remote as 50-70° from the critical point. It is highly improbable that the errors in density and surface tension measurements in this temperature range would be sufficiently large and systematic to account for the very definite and progressive increase in the parachors of propylene, benzene, and chlorobenzene, whereas in the cases of methyl ether and carbon tetrachloride no such increase is evident.

Owing to the unfortunate lack of sufficient data from which to draw definite conclusions, it is not possible to advance a general explanation with a large degree of certainty. From the few data available, however, it would seem that those molecules which possess a multiple bond in their structure, as a consequence of which considerable electronic interaction might be expected, show an increase in parachor with temperature. On the other hand, those molecules which possess no multiple linkages in their constitution do not exhibit a tendency towards an increase in parachor with a rise of temperature. In this latter type of molecule, little or no electronic interaction would be expected.

The increase in parachor due to the presence of double or triple bonds, or to ring systems, has been accounted for on the basis of electronic interaction. It postulated that, as a result of this interaction where the electrons are probably congested, as in the case of a double bond, the tendency is towards an expansion of the molecule. This is to be regarded as analogous to an increase in parachor, since evidence points to a direct proportionality between the parachor of a molecule and its range of molecular action, which is of the same order of magnitude as the molecular diameter.

By a similar argument, it is conceivable that an increase in temperature would result in an increased vibrational energy of the molecule, in an increased electronic interaction, and thus in an increased sphere of molecular influence. This would be evident in those molecules in which electronic interaction is pronounced, whereas, in the case of molecules containing only single bonds, and consequently negligible interaction between the electrons, the coefficient of parachor increase with temperature would also be negligible. In the case of the former type of molecule, the increased molecular action would be reflected in an increased parachor. This, in turn, might be interpreted as an increase in unsaturation, since expansion of the molecule, due to augmented electronic interaction, would increase the tendency of the unsaturated carbon atoms to share electrons.

### *B. Ring Method*

In determining the utility of this method for the determination of surface tension near the critical temperature, the surface tension-temperature relations for methyl ether have been investigated over a range of temperature from 111.5°C. to 119.2°C. A curve of the extensions of the spiral observed at five different temperatures was first plotted. Reference was then made to

the curve obtained for the surface tension of methyl ether by the capillary rise method, and the surface tension at 111.5°C. ascertained. Using the well-known formula for the ring method,  $T = \frac{mg}{2L}$ , where  $T$  is the surface tension of the liquid,  $m$  is the force in grams necessary to remove the ring from the surface,  $g$  is the gravity constant, and  $L$  is the circumference of the ring, the value of  $L$  was calculated by substitution of the value of  $T$  at 111.5°C. Then, since  $m$  corresponding to any other temperature could be ascertained from the observed extension of the calibrated quartz spiral, the value of the surface tension at any other temperature in the range studied was readily calculable.

In Table V is shown the agreement between the values obtained with the ring method and those read from the curve obtained by the capillary rise method, for two-degree intervals between 110.0°C. and 120.0°C., a small extrapolation being necessary to obtain these two particular values. The values show remarkably good agreement, even at the higher temperatures.

TABLE V

COMPARISON OF THE SURFACE TENSIONS OF METHYL ETHER BY THE RING AND CAPILLARY RISE METHODS

Temp., °C.	110.00	112.00	114.00	116.00	118.00	120.00
S.T. (ring), dyne/cm.	1.16	1.04	0.89	0.71	0.54	0.39
S.T. (cap. rise), dyne/cm.	1.21	1.04	0.88	0.70	0.54	0.38

Although the data are not comprehensive, they serve to indicate two points of significance. The ring method is independent of the angle of contact which the liquid makes with the glass. The results obtained by the capillary rise method were calculated on the assumption that the angle of contact was zero. Since these results agree with those obtained by the ring method, it must be concluded that the angle of contact between methyl ether and glass is zero. Furthermore, although the ring method was abandoned in favor of the capillary rise method, purely from the standpoint of manipulative ease, the results obtained by the former method do indicate the feasibility of applying this method to the determination of surface tension near the critical point. With some modifications, it is conceivable that the method would be much more readily applicable than in its present form. That the ring method possesses certain advantages for work in the critical region is undoubtedly true. Should it prove to be the case that a meniscus persists above the critical point, although optically undetectable, the ring method, or a modification thereof, would seem to be the most suitable method for its detection, and for the determination of the surface energy involved. The fact that the method is independent of the angle of contact is also an advantage, as is also the fact that the densities of the liquid and vapor phases need not be known.

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## THE YELLOW COLORING MATTER OF KHAPLI WHEAT, *TRITICUM DICOCCUM*

### III. THE CONSTITUTION OF TRICIN<sup>1</sup>

BY J. ANSEL ANDERSON<sup>2</sup>

#### Abstract

Tricin is identical with 5,7,4'-trihydroxy-3',5'-dimethoxyflavone which has been synthesized by the action of concentrated sulphuric acid on 5,7-dihydroxy-3',4',5'-trimethoxyflavone. The constitution has been confirmed by fusion with alkali which yielded syringic acid and phloroglucinol.

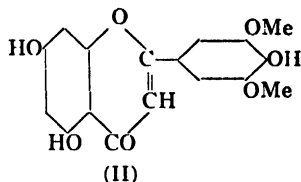
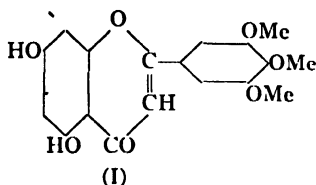
The isolation of tricin, a trihydroxydimethoxyflavone, from Khapli wheat leaves was reported by Anderson and Perkin (2). In a later communication (1) it was shown that tricetin, the compound formed by demethylation of tricin, was 5,7,3',4',5'-pentahydroxyflavone. In view of the weak dyeing properties of tricin as compared with those of tricetin, it at once appeared altogether likely that in the former, two of the three hydroxyl groups in the 3'-, 4'- and 5'-positions were methylated. In these circumstances it seemed most probable that tricin contained the syringic nucleus, which occurs quite commonly in compounds of natural origin. The possibility that the methoxyl groups occurred in the 3'- and 4'-positions was, however, by no means negligible since this condition occurs in the isoflavone, irigenin. Synthetic experiments designed to determine the constitution of tricin were not undertaken immediately since the isolation of a further supply of tricin was expected during the examination of a fresh supply of wheat leaves, thus making it possible to proceed by the more logical method of determining the hydrolyzation products of tricin before attempting its synthesis. In the meantime, Venkataraman and Gulati have formed the opinion that tricin contains the syringic nucleus and have published a note (5) declaring their intention of synthesizing 5,7,4'-trihydroxy-3',5'-dimethoxyflavone by the interaction of phloracetophenone and O-benzylsyringic anhydride.

Approximately one gram of fairly pure tricin was isolated recently from Khapli wheat leaves. Attempts to hydrolyze the compound with potassium hydroxide in 80% alcohol failed owing to the relative insolubility of the potassium salt in this medium. The hydrolysis of the small quantity of tricin remaining after the completion of the above experiments, was effected by the use of 50% alcohol, in which the compound remained dissolved. The results were by no means clearcut but offered some indications that tricin contained the syringic nucleus and was therefore tricetin 3',5'-dimethyl ether (II).

<sup>1</sup> Manuscript received July 4, 1933.

Contribution from the National Research Laboratories, Ottawa, Canada.

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Synthetic experiments were undertaken pending the isolation of more tricin. A quantity of 5,7-dihydroxy-3',4',5'-trimethoxyflavone (I) being available, an attempt was made to demethylate the group in the 4'-position by means of sulphuric acid under conditions similar to those used for the preparation of syringic acid from 3,4,5-trimethoxybenzoic acid (3, p. 1553). The first attempt proved successful and slight variation of the conditions in later experiments led to a fair yield of tricetin 3',5'-dimethyl ether (II). Heap and Robinson (4) attempted the preparation of syringetin by this method but were unable to find the conditions for its successful use.

5,7,4'-Trihydroxy-3',5'-dimethoxyflavone (II) proved to be identical with tricin in all respects. Since tricin had not proved amenable to alkaline hydrolysis, confirmation of the structure was attempted by fusion with alkali. The methoxyl groups were quite stable under the conditions employed and syringic acid and phloroglucinol were isolated.

The water-soluble coloring matters of wheat leaves are still under investigation and the examination of other organic constituents of the plant has also been undertaken.

### Experimental

#### 5,7,4'-Trihydroxy-3',5'-dimethoxyflavone (*Tricin*)

5,7-Dihydroxy-3',4',5'-trimethoxyflavone (1) (3 gm.) was dissolved in sulphuric acid (4.5 cc. of 96-98%) and heated at 60° C. A small quantity of orange precipitate separated gradually. After 6 hr. the mixture was transferred to water (200 cc.). The orange-yellow, lumpy precipitate which separated was broken up and the mixture was heated on the steam bath for 0.75 hr. The solid was collected, washed with water and crystallized from hot dilute acetic acid (1.43 gm.), m.p. 288-289° C.\* Three recrystallizations from acetic acid yielded clusters of pale yellow, glistening needles, m.p. 291-292° C. Analysis: Calcd. for  $C_{17}H_{14}O_7$ ; C, 61.8; H, 4.3;  $CH_3O$ , 18.8%. Found after drying: C, 61.6; H, 4.3;  $CH_3O$ , 18.3%.

Previous preparations of natural tricin had softened and melted indefinitely over a comparatively wide range. Some trouble was experienced in preparing a sample melting sharply at as high a temperature as the synthetic specimen. This was finally effected by careful deacetylation of the pure acetyl derivative and recrystallization from acetic acid. A mixture of equal quantities of the synthetic and natural compounds melted at 291-292° C.

5,7,4'-Trihydroxy-3',5'-dimethoxyflavone is fairly readily soluble in ethyl alcohol, methyl alcohol and acetic acid; soluble in ethyl acetate; rather

\*All melting points are corrected.



sparingly soluble in ether; very sparingly soluble in chloroform and toluene; almost insoluble in benzene and water; and insoluble in ligroin. It dissolves in concentrated sulphuric acid and in dilute aqueous sodium hydroxide to give bright yellow solutions. In alcoholic solution it gives a reddish-brown color with a trace of alcoholic ferric chloride and a dark olive-brown with more concentrated reagent. With alcoholic lead acetate it gives a bright yellow precipitate and with alcoholic potassium acetate a pale yellow precipitate. No color changes could be obtained by treating an alcoholic solution of the flavone with very dilute alcoholic potassium hydroxide. A hot concentrated solution of the flavone in glacial acetic acid, when treated with a trace of concentrated sulphuric acid and cooled, yielded clusters of minute, bright orange needles of the oxonium salt. The coloring matter is a very weak dye. It gives a pale lemon-yellow on aluminium-, a pale brown on iron-, and a greenish-yellow on chromium-mordanted wool. These properties are identical with those of tricin.

#### *5,7,4'-Triacetox-3',5'-dimethoxyflavone*

5,7,4'-Trihydroxy-3',5'-dimethoxyflavone (0.15 gm.) was refluxed for 1 hr. with acetic anhydride (0.6 cc.) containing a trace of pyridine. The acetyl compound crystallized out on cooling (0.16 gm.). It was recrystallized from acetic anhydride and again from a mixture of alcohol and acetic acid and was thus obtained in colorless needles, m.p. 251-254° C. Analysis: Calcd. for  $C_{23}H_{20}O_{10}$ : C, 60.5; H, 4.4;  $CH_3O$ , 13.6%. Found: C, 60.6; H, 4.5;  $CH_3O$ , 13.7%. The melting point was unchanged when the compound was mixed with an equal quantity of triacetyl tricin.

#### *Alkaline Fusion*

5,7,4'-Trihydroxy-3',5'-dimethoxyflavone (1.5 gm.) was heated for 5 min. at 200-210° C. with potassium hydroxide (15 gm.) and a few drops of water. The melt was dissolved in water and acidified with hydrochloric acid. After filtering from a trace of dark precipitate the solution was thoroughly extracted with ether. The residue which remained after evaporation of the ether was taken up in a solution of sodium bicarbonate, filtered from a trace of yellow insoluble matter, and again extracted with ether (A).

The extracted aqueous solution was acidified with dilute hydrochloric acid. A crystalline precipitate (B) of almost colorless needles separated immediately. This was collected and the filtrate was again extracted with ether (C).

*Isolation and identification of phloroglucinol.* The ether extract (A) was dried and evaporated to dryness. The residue was taken up in a little water, filtered from a minute trace of insoluble matter and the filtrate evaporated to dryness *in vacuo*. A crystalline residue remained (0.12 gm.), m.p. 216-218° C. A small quantity of this compound when dissolved in water gave a bluish-violet color with aqueous ferric chloride solution.

The compound (0.1 gm.) was dissolved in a solution of potassium hydroxide (0.155 gm.) in water (0.5 cc.). The solution was cooled in melting ice and a

small piece of ice was added. Acetic anhydride (0.24 cc.) was then added with vigorous shaking. An almost colorless precipitate separated immediately. It was collected, washed with water and dried (0.11 gm.). Recrystallization from alcohol yielded minute, colorless needles (0.07 gm.) which melted at 105-106° C. and at the same temperature when mixed with an equal quantity of triacetyl phloroglucinol.

*Isolation and identification of syringic acid.* The precipitate (B) was washed with a little water and dried (0.43 gm.), m.p. 208-209° C. It was recrystallized from water (Norit) and was thus obtained in colorless, silky needles which melted at 210-211° C. and at the same temperature when mixed with an equal quantity of syringic acid.

The ether extract (C) was dried and evaporated to dryness. The residue (0.18 gm.) by recrystallization from water yielded a small quantity of syringic acid. Color reactions indicated the presence of a trace of gallic acid.

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# ON THE SPECIFIC HEAT OF COPPER FROM $-78^{\circ}$ TO $0^{\circ}$ C.<sup>1</sup>

By S. M. DOCKERTY<sup>2</sup>

## Abstract

This paper is a continuation of recent work by H. L. Bronson, H. M. Chisholm, and the author (3) on the specific heats of tungsten, molybdenum, and copper from  $0^{\circ}$  to  $500^{\circ}$  C. The "method of electrical heating" and adiabatic calorimetry have been extended to determine the specific heat of copper from  $-78^{\circ}$  to  $0^{\circ}$  C.

The equation previously given for the specific heat of copper contained only the first two terms of the Debye expansion and was found not to hold below  $-30^{\circ}$  C. The following equation containing four terms of the Debye expansion fits the experimental curve from  $-78^{\circ}$  to  $500^{\circ}$  C. with a maximum deviation of only about 0.05%.

$$C_p = .3889 + 5.65 \times 10^{-5} T - \frac{2000}{T^2} \left( 1 - \frac{321^2}{287^2} + \frac{321^4}{9107^4} \right),$$

where the units are joules per gram per  $^{\circ}$ K. The constants of this equation were determined empirically and their close relation to theoretically expected values has been discussed.

## Introduction

In the previous work an adiabatic calorimeter of the Richards' type was used except that the usual water calorimeter was replaced by solid copper calorimeters from 1 to 5 kgm. in mass. The specific heat of copper from  $-5^{\circ}$  to  $110^{\circ}$  C. was determined directly by heating these calorimeters electrically under adiabatic conditions. The current and voltage were measured with a potentiometer, and the rise in temperature with a platinum thermometer and bridge of the Callendar-Barnes type (1). The "copper equivalent" of each calorimeter was accurately known since the non-copper components were only about 0.5%. All measurements were thus made directly and with high precision; the method is therefore practically free from the errors and difficulties experienced in the use of the "method of mixtures." It also has the great advantage of giving the average specific heat over a small range of temperature and is particularly well suited for the measurement of specific heat at, or near, room temperature where the "method of mixtures" is quite unsuitable. The slope of the specific heat-temperature curve thus obtained increased more and more rapidly as the temperature decreased to  $0^{\circ}$  C. This is of particular interest from a theoretical standpoint and it seemed desirable to extend this accurate method of measurement to still lower temperatures.

In the present investigation it has been found possible to overcome the difficulties of adiabatic calorimetry at temperatures down to  $-78^{\circ}$  C., and the precision of the results obtained compare favorably with those obtained above room temperature in the previous investigation (3).

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## Apparatus and Experimental Method

### Adiabatic Calorimeter

It was not thought practicable with the apparatus available to use a liquid bath for the low temperature work, so a thick cast-copper shell was made to replace the oil bath and jacket of the adiabatic calorimeter used in the previous experiments. This shell (*A*, Fig. 1) was made of such dimensions that it was suitable for the accommodation of the 1100-gm. copper calorimeter used in the previous investigation. The body and bottom are in one piece, while between the cover and body there is a carefully ground joint. Three separate heating coils were wound in grooves on the sides and on the ends and the whole was thermally insulated from the outside by cotton wool packing between the shell and the container (*D*, Fig. 1).

It was hoped that the high conductivity of copper and the thickness of the shell would ensure proper adiabatic conditions over the inner surface of the shell even when there was considerable variation in temperature over the outer surface. This however was not found to be the case and variations were present of a size which indicated that the shell, which was not of pure copper, had a conductivity considerably less than was expected. It was therefore necessary to adjust the heating and thermal insulation on the outside of the shell with considerable care.

Differences in temperature between points on the inside of the shell were measured by means of a thermocouple, the junctions of which could be moved from point to point over the inner surface.

Temperature inequalities on the inner surface between sides, bottom, and top, caused by faulty distribution of heat losses, were largely eliminated by adjusting the amount of thermal insulation on the sides and ends. This was accomplished for the ends by adjusting the spaces between the top and bot-

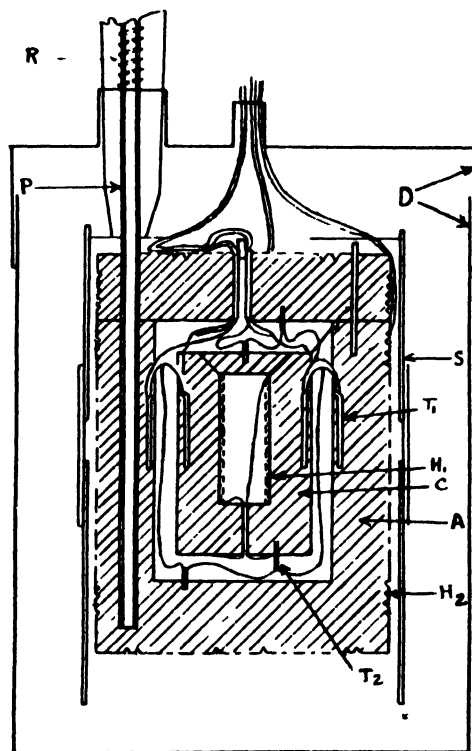


FIG. 1. Adiabatic calorimeter assembled.

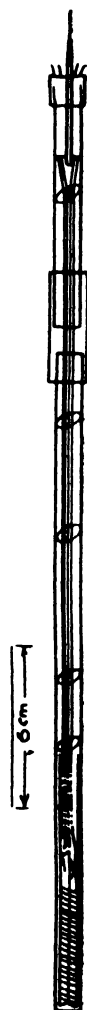


FIG. 2. Platinum thermometer.

tom of the shell and the container, and for the sides by varying the length of the brass shield (*S*, Fig. 1) and thus changing the effective area of the sides. These adjustments were successfully made when there was a temperature difference of 90° C. between the shell and the air of the room. The heat loss to the outside for such a temperature difference was the equivalent of about 20 watts. The fraction of the current in each heating coil was then so adjusted that uniformity in temperature over the inner surface was also obtained when the shell was rising in temperature at the rate of 0.25° C. per minute (the usual rate during an experiment).

### *The Copper Specimen*

The specimen of copper used in this work was the 1100-gm. cold-rolled copper calorimeter used in the previous experiments, and has the form and dimensions shown in *C*, Fig. 1. As the calorimeter was 99.5% copper there was no difficulty in calculating the "copper equivalent" to the required degree of accuracy. The weights of the various parts and their calculated "copper equivalents" are given in Table I.

TABLE I  
WEIGHTS OF CALORIMETER PARTS AND THEIR CALCULATED COPPER EQUIVALENTS

Part of calorimeter	Weight, gm.	Copper equiv., gm.	Part of calorimeter	Weight, gm.	Copper equiv., gm.
Copper calorimeter	1128.95	1128.95	Aluminium foil	0.04	0.10
Brass hinges and screws	4.10	4.04	Copper cylinder for heating coil	15.37	15.37
Steel supporting wires	0.03	0.04	Manganin wire for heating coil	2.54	2.63
Brass tubing for thermocouples	2.43	2.40	Cotton insulation for heating coil	0.965	3.92
Wire for thermocouples	0.16	0.16	Copper leads for heating coil	0.46	0.46
Cotton insulation on thermocouples	0.15	0.60	Air correction		0.40
Cement for thermocouples	0.15	0.30	Total		1159.4

Although there is some uncertainty in the "copper equivalent" of the cotton insulation, an error of as much as 0.5 gm. is highly improbable.

The thermocouple, current, and potential leads were brought out through a hole in the top of the shell and were run for some distance along its top to prevent heat losses from the inner calorimeter along these wires.

### *Differential Thermocouples*

Temperature equality between the calorimeter and the shell was assured by an eight-junction thermocouple of No. 34 copper and No. 32 constantin wire. The junctions of this thermocouple were set in plastic cement in brass tubes and were arranged in two pairs of three-junction and two pairs of one-

junction couples. These were mounted on the sides and ends of the calorimeter and shell in the ratio 6 : 1 : 1. Since this was about the ratio of the areas involved the arrangement should have largely eliminated the effects of any temperature differences on the inner surface of the shell. Opposite junctions were similarly mounted ( $T_1$  and  $T_2$ , Fig. 1) in order to eliminate the effects of lag and of possible discrepancies arising from the shortness of some of the junctions. With a sensitive galvanometer this thermocouple gave a deflection of 8 mm. for a temperature difference of  $0.001^\circ \text{C.}$  between the calorimeter and shell.

### *Measurement of Temperature*

The temperature of the copper calorimeter was found by measuring the temperature of the shell when the differential thermocouples indicated temperature equality between the two. This was done by means of a platinum thermometer of the form shown in Fig. 2. This thermometer had a resistance of 20 ohms at  $0^\circ \text{C.}$  and the sensitivity of the bridge was such that settings could be made with confidence to within  $0.001^\circ \text{C.}$  The upper part of the thermometer was of Pyrex glass and was joined by a piece of heavy rubber tubing to the nickel stem. This nickel tube was 8 mm. in diameter and fitted neatly into a hole 14 cm. deep in the copper shell ( $P$ , Fig. 1). The exposed stem was carefully wrapped with cotton wool to prevent heat losses from the shell along the metal stem. In spite of this, however, it was found that when the temperature of the shell was considerably above that of the room enough heat was conducted up the stem to upset the thermal adjustments previously made. This difficulty was overcome by winding a heating coil ( $R$ , Fig. 1) on the upper part of the nickel stem and adjusting the heating current till the thermal adjustments were the same as they were before the thermometer was inserted. A curve was drawn giving the required value of this current for any particular temperature difference between the shell and the outside surroundings.

### *Constants of the Platinum Thermometer*

In using the platinum thermometer for the measurement of temperature the following equations are internationally accepted (4):

$$R_t = R_0(1 + At + Bt^2) \dots\dots\dots 0^\circ \text{ to } 660^\circ \text{C.}$$

$$R_t = R_0(1 + At + Bt^2 + C(t - 100)t^3) \dots\dots\dots 0^\circ \text{ to } -190^\circ \text{C.}$$

where  $t$  is in  $^\circ \text{C.}$  If  $\frac{R_t - R_0}{R_{100} - R_0} \times 100$  is denoted by  $t_p$  we obtain equations in the usual convenient form for temperature calculation:

$$t - t_p = \delta \left( \frac{t_p^2}{100^2} - \frac{t_p}{100} \right) \dots\dots\dots 0^\circ \text{ to } 660^\circ \text{C.}$$

$$t - t_p = \delta \left( \frac{t_p^2}{100^2} - \frac{t_p}{100} \right) + \gamma(t - 100)t^3 \dots\dots\dots 0^\circ \text{ to } -190^\circ \text{C.}$$

The constants  $\delta$  and  $\gamma$ , determined from the boiling points of water, sulphur, and liquid oxygen, were found to be:— $\delta = 1.52$ — $\gamma = 9.3 \times 10^{-10}$ .

*Preliminary Experiments Above Room Temperature*

Before proceeding to the low temperature measurements it was considered desirable to test the apparatus over a range which had been previously investigated. Specific heat determinations were therefore made at 10° intervals from 20° to 110° C. with the apparatus in open air. The experimental procedure for such measurements has been fully discussed in the previous paper (3). No difficulty was found in maintaining temperature equality between the calorimeter and jacket to within 0.003° C. while the calorimeter was being heated and calculation showed that a difference of 0.01° during the whole time of an experiment would introduce an error of less than 0.1%. At the end of an experiment not less than 15 min. was allowed for the copper shell to reach equilibrium before the platinum thermometer was read. During these determinations the platinum thermometer stem was heated in the manner previously discussed. The results obtained are given in Column 2, Table II. In Column 3 are shown the results previously obtained when an oil bath was used. In Column 5 are values of  $I^2$ , the mean of the squares of the heating currents necessary to maintain the shell at a constant temperature at the beginning and end of an experiment.

TABLE II  
SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Mean temp., °C.	Specific heat		Diff.	$I^2$	Correction	Corr. value
	Experimental	Former results				
25.50	0.3842	0.38415	0.00005	0.007	0.00005	0.38415
36.58	0.3863	0.3863	0.00000	0.020	0.0001	0.3862
47.58	0.38835	0.38825	0.00010	0.035	0.00015	0.3882
58.52	0.39035	0.39005	0.00030	0.050	0.0002	0.39015
69.40	0.3920	0.39175	0.00025	0.064	0.00025	0.39175
80.24	0.3937	0.3933	0.00040	0.078	0.0003	0.3934
91.02	0.3952	0.39485	0.00035	0.095	0.0004	0.3948
101.75	0.3967	0.39625	0.00045	0.110	0.00045	0.39625
*105.17	0.39715	0.3967	0.00045	0.098	0.0004	0.39675
106.20	0.3974	0.39685	0.00055	0.118	0.0005	0.3969
*115.91	0.39845	0.3980	0.00045	0.110	0.00045	0.3980

\*Taken after low temperature determinations.

It is evident from the above figures that the deviation of the present experimental values from the former results is approximately proportional to  $I^2$ . The corrections in Column 6 are calculated as follows:—

Correction =  $\frac{I^2 \times 0.00045}{0.110}$ . This is of course done on the assumption that

the original experimental curve was correct and that the differences in Column 4 are due to inequalities in temperature over the inner surface of the shell. Such inequalities would be proportional to the heat losses from the shell at any temperature and hence to  $I^2$  which is a measure of these losses. Further

confirmation of the fact that the differences of Column 4 are real and that the observed values should be corrected as above will be shown later in connection with the low temperature measurements. Undoubtedly these corrections could be reduced by readjustment of the heating and thermal insulation, but it did not seem worth while when the maximum correction was only about 0.1%.

#### THE SPECIFIC HEAT OF COPPER BETWEEN $-78^{\circ}$ AND $30^{\circ}\text{C}$ .

For the low temperature work the apparatus was placed in a thermally insulated receptacle and solid carbon dioxide was packed around the container *D*. The shell was then cooled rapidly to  $-120^{\circ}\text{C}$ . by means of liquid air so that the temperature of the inner calorimeter was lowered to  $-78^{\circ}\text{C}$ . in about one hour. When this point was reached the temperature of the calorimeter and shell were made equal and the platinum thermometer read. Specific heat determinations were then made, as before, at  $10^{\circ}$  intervals from  $-78^{\circ}$  to  $30^{\circ}\text{C}$ . The time required for these measurements was only about 11 hr., which indicates the convenience of the method. The apparatus was about as easy to operate at this temperature as when working above room temperature. After the carbon dioxide had evaporated two determinations were made above  $100^{\circ}\text{C}$ . without disturbing the apparatus. These two points (marked with an asterisk in Table II) are in complete agreement with those obtained when the apparatus was in the open air. Since the surroundings of the container and of the exposed stem of the platinum thermometer were maintained at  $-78^{\circ}\text{C}$ . during the low temperature experiments, the thermal conditions were similar to those obtained when working above room temperature with the container in the open air. The corrections in Table II should therefore be equally applicable to the lower range.

In Column 2 of Table III are given the values obtained for the specific heat of copper at various temperatures from  $-78^{\circ}$  to  $30^{\circ}\text{C}$ ., while in Column 3 are the corresponding values from the experimental curve of the previous

TABLE III  
SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Mean temp., $^{\circ}\text{C}$ .	Specific heat		$I^2$	Correction	Corr. value
	Experimental	Former results			
$-72.00$	0.35495		0.012	0.00005	0.3549
$-60.70$	0.3599		0.027	0.0001	0.3598
$-50.11$	0.3641		0.035	0.00015	0.36395
$-38.90$	0.36775		0.044	0.00017	0.3676
$-27.30$	0.37145		0.055	0.00022	0.37125
$-16.50$	0.37465		0.065	0.00025	0.3744
$-5.80$	0.37745	0.37715	0.073	0.0003	0.37715
5.54	0.38005	0.3799	0.084	0.00033	0.3797
16.08	0.3826	0.38225	0.093	0.00037	0.38225
25.84	0.38465	0.38425	0.102	0.0004	0.38425



investigation (3). Columns 4 and 5 contain the values of  $I^2$  and of the corrections calculated exactly as in Table II. The fact that the corrected values around room temperature agree, within the limits of experimental error, with those of Column 3 and also with the lowest value in Column 2, Table II, would seem to justify the method of calculating the corrections.

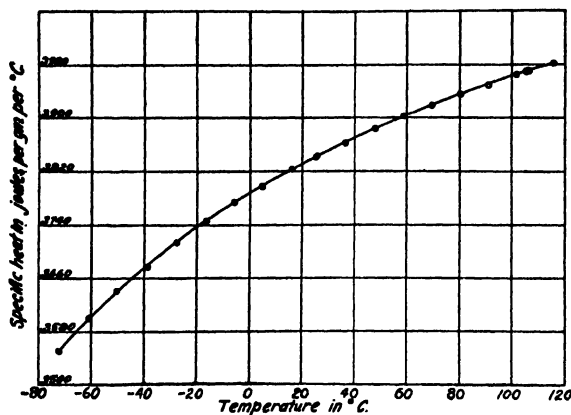


FIG. 3. *Specific heat of copper.*

copper shell is a satisfactory substitute for the bath and jacket of the calorimeter previously used. There is nothing to indicate that the results obtained from  $-78^{\circ}$  to  $0^{\circ}$  C. are less accurate or less reliable than those for the range  $0^{\circ}$  to  $120^{\circ}$  C. It would seem, therefore, that this type of apparatus might be successfully used for accurate specific heat determinations at still lower temperatures.

### Equations for the Specific Heat of Copper

In the previous paper (3) it was pointed out that theoretical considerations suggested that an equation for the specific heat of metals should have the form:

$$C_p = A + BT - \frac{C}{T^3} \quad (a)$$

where  $T$  is in degrees  $K$ . The first and last terms represent the first two terms of the Debye (5) expansion for  $C_p$  while the middle term represents  $C_p - C_v$ . The constants of this equation for the same copper calorimeter as used in the present experiments were determined empirically with the following results:

$$C_p = .3884 + 5.69 \times 10^{-3}T - \frac{1890}{T^3} \quad (9J)^*$$

in which the units are joules per gram per degree  $K$ . This equation fitted the experimental points from  $-5^{\circ}$  to  $110^{\circ}$  C. with an average deviation of less than  $0.05\%$ . The equations obtained for the specific heat of other specimens of copper, even up to  $500^{\circ}$  C., differed from Equation (9J) only

\*This equation number used in the previous paper.

When the values for the specific heat of copper given by the last columns of Tables II and III were plotted to a large scale only one point was found to lie off a smooth curve by as much as 1 part in 2000. This curve on a much smaller scale is shown in Fig. 3.

A comparison of the results obtained from  $-5^{\circ}$  to  $110^{\circ}$  C. using the present type of adiabatic calorimeter with those obtained in the previous investigation indicates that the

in the first term and there by not more than 0.2%. We may therefore conclude that the *experimental* values of the specific heat of the copper calorimeter between 0° and 500° C. can be calculated from Equation (9J). Variations of a few tenths of 1% in the specific heat of different samples of copper are not surprising in the light of recent work by Jaeger, Rosenbohm, and Bottema (7), who have found that the specific heats of annealed and unannealed samples of both platinum and copper differed considerably, the former by as much as 2%.

In Column 2 of Table IV are shown the values of the specific heat of copper from -78° to 120° C. taken from the present experimental curve while Column 3 shows the values calculated by Equation 9J from -78° to 500° C. A comparison of Columns 2 and 3 shows that Equation 9J and the experimental curve are in very close agreement down to 250° K. but that they diverge rapidly below this point.

An examination of the magnitude of successive terms of Debye's (5) expansion:

$$C_v = C_\infty - \frac{C_\infty \theta^2}{207^2} + \frac{C_\infty \theta^4}{5607^4} - \frac{C_\infty \theta^6}{181447^6} + \dots$$

indicates that for temperatures of 200° K. the third and even the fourth term cannot be neglected and that Equation (a) should have the form:

$$C_p = C_\infty + BT - \frac{C_\infty \theta^2}{207^2} \left( 1 - \frac{\theta^2}{287^2} + \frac{\theta^4}{9107^4} \right) \quad (b)$$

The constants of this equation were determined empirically from the value of  $C_p$  at 200° K. given in Column 2, and the values at 400° and 800° K. given in Column 3 with the following results:

$$C_p = 0.3889 + 5.65 \times 10^{-7} T - \frac{2000}{T^2} \left( 1 - \frac{321^2}{287^2} + \frac{321^4}{9107^4} \right) \quad (1)$$

The values calculated by this equation (Column 4, Table IV) agree closely with those in Column 3 above 300° K., while the maximum deviation from the experimental values of Column 2 is only about 0.05% at 250° K. Although Equation (1) fits the experimental curve remarkably well over a wide temperature range it is not entirely satisfactory from a theoretical standpoint. It was assumed that  $C_p - C_v$  could be represented by a term  $BT$ , whereas thermodynamic theory indicates that it should have the form  $\frac{9\alpha^2 V}{K} T$ ,

where  $V$  is the specific volume and  $K$  the compressibility, both of which vary little with the temperature;  $\alpha$  is the coefficient of linear expansion which Grüneisen (6) has shown to be directly proportional to  $C_v$ . Thus  $C_p - C_v$  should have the form  $GC_v^2 T$ . Since the ratio of  $C_p$  to  $C_v$  is approximately constant, and since  $C_p$  is the quantity determined experimentally, it is more convenient to express  $C_p - C_v$  as  $G'C_p^2 T$  which is the form commonly given in standard text books. For copper the value of  $G'$  in such cases is given as  $1.3 \times 10^{-6}$  if the units are calories per mole, or  $2.0 \times 10^{-4}$  if the units are joules per gram. If  $C_v$  is used in the above formula instead of  $C_p$ , and the ratio of  $C_p$  to  $C_v$  taken as 1.025 the constant  $G$  becomes  $2.1 \times 10^{-4}$ .

Born and Brody (2) have shown that Debye's expression for  $C_v$  should contain a term  $DC_vT$  due to the anharmonic oscillations of the atoms, and according to Sommerfeld (8) the effect of free electrons in the metal requires still another term of the form  $ET$ . The sum of these two terms may be represented with sufficient accuracy by a single term  $HT$ , since  $DC_v$  above is very small and nearly constant. The following equation therefore would seem to have a fairly sound theoretical basis:

$$C_p = C_\infty + HT + GC_p^2T - \frac{C_\infty \theta^2}{20T^2} \left( 1 - \frac{\theta^2}{28T^2} + \frac{\theta^4}{910T^4} \right) \quad (c)$$

Since the second and third terms of this equation are both small and the third term is nearly proportional to  $T$ , it is obviously impossible to evaluate the constants  $H$  and  $G$  separately with any degree of accuracy. It was decided, therefore, to adopt for  $G$  the value given above and solve for the three remaining constants. Using the same data as in the case of Equation (1) the following equation was obtained:

$$C_p = 0.3911 + 1.95 \times 10^{-5}T + 2.1 \times 10^{-4}C_p^2T - \frac{1998}{T^2} \left( 1 - \frac{320^2}{28T^2} + \frac{320^4}{910T^4} \right) \quad (2)$$

The values calculated from this equation are shown in Column 5 of Table IV. Although Equations (1) and (2) are seen to fit the experimental curve equally well, it would seem that the constants of the latter had decidedly more theoretical significance.

As a matter of interest, and in order to see how the various constants would be affected, Equation (3) was obtained in which  $G'C_p^2T$  was used in place of  $GC_p^2T$  above.

$$C_p = 0.3938 + 1.34 \times 10^{-5}T + 2.0 \times 10^{-4}C_p^2T - \frac{2060}{T^2} \left( 1 - \frac{323^2}{28T^2} + \frac{323^4}{910T^4} \right) \quad (3)$$

The values calculated from this equation (Column 6, Table IV) also agree closely with the experimental points, but the slope of the experimental curve at 250° K. differs from that given by Equation (3) by more than the errors of measurement, which indicates that Equation (2) is preferable to Equation (3). For convenience the values for  $C_p$  calculated from data given in

TABLE IV  
SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE K.

Temp., °K.	Experi- mental	Eq. (9J)	Eq. (1)	Eq. (2)	Eq. (3)	Value of $C_p$ from Eq. (2)
200	0.3544	0.3525	0.3544	0.3544	0.3544	0.3493
225	0.3646	0.3639	0.3648	0.3647	0.36495	0.3586
250	0.3725	0.3724	0.37275	0.3728	0.3730	0.3658
275	0.3791	0.3790	0.3792	0.3791	0.3795	0.3712
300	0.3845	0.3845	0.38455	0.3845	0.38475	0.3756
325	0.3890	0.3890	0.3890	0.3890	0.3892	0.3792
350	0.3929	0.3929	0.3929	0.3929	0.3930	0.3822
375	0.3964	0.3963	0.3963	0.3962	0.3964	0.3845
400		0.3993	0.3993	0.3993	0.3993	0.3867
500		0.4093	0.4093	0.4092	0.4091	0.3930
600		0.4173	0.4173	0.4173	0.4171	0.3974
700		0.4244	0.4244	0.4243	0.4242	0.4007
800		0.4310	0.4310	0.4310	0.4310	0.4036

Equation (2) are given in the last column of Table IV. It should again be pointed out that between 270° and 800° K., Column 3 represents the *experimental* results of the previous investigation (3).

### Discussion of Equations

According to the Debye theory the value of  $C_\infty$  in these equations should equal  $3R$  per mole or, in the case of copper, 0.3922 when the units are joules per gram. In none of the above equations does the constant term differ from this value by as much as 1% while in the case of Equation (2) the difference is less than 0.3%. The values of  $\theta$ , the "characteristic temperature" of copper, obtained from Equations (1), (2) and (3) are 321°, 320°, and 323° K. respectively, which agree closely with the value 321° K. found from the  $T^3$  Law. If the values of  $C_\infty$  or of  $\theta$  in any one of these equations be slightly changed it is in general possible to adjust the other constants so that the new equation will fit the experimental curve about as well as the original, but the limits for such changes are decidedly less than 0.5%. Obviously greater accuracy and more certainty for these constants could be obtained by extending the range of temperature to still lower values, where the second and third terms of Equation (c) would be smaller and the terms containing  $\theta$  would be larger.

A comparison of Equations (2) and (3) shows that the empirically determined value of  $H$  varies considerably, depending on the nature and magnitude of the term chosen for  $C_p - C_v$ , so that the value given in Equation (2) can be regarded only as a rough estimate which can be improved only by a more accurate knowledge of the term representing  $C_p - C_v$ . However, a term of this order of magnitude seems to be required theoretically and its inclusion in the Debye expansion is definitely necessary if the equation is to fit the experimental curve at high temperatures.

Equation (1) rather than the theoretically more exact Equation (2) has been selected for the abstract because it is more useful for purposes of calculation.

### Acknowledgment

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## SECULAR CHANGES OF THE MAGNETIC ELEMENTS, OTTAWA, 1500-1930<sup>1</sup>

By W. H. HERBERT<sup>2</sup>

### Abstract

In this paper is presented a table of the various elements of terrestrial magnetism at Ottawa from 1500 to 1930 and explains how the values were derived from old magnetic observations made in America, and not from theory. Among other points, it shows that though the total magnetic force has been declining at Ottawa for some time, yet the total magnetic force and the magnetic elements evidently go through cycles and none have apparently suffered permanent change during the time considered.

Original tables covering the secular change of declination for many places in Canada, 1750-1925, were compiled and published in 1926 by the Topographical Survey, Department of the Interior, in Bulletin 58: The March of the Compass in Canada. The main objects of those tables were to enable surveyors to retrace old lost land boundaries run by compass many years ago, and to afford this Survey a ready means of keeping up-to-date its more than 30,000 field measurements of declination made throughout the Dominion during the past half-century for the purpose of compiling every five years its declination chart of Canada—the only such chart published for this country.

In compiling those tables, the writer became interested in the wider question as to how far back the secular change of declination in Canada might be extended, and started to investigate the subject, soon discovering, however, that the time consumed in sifting over a large number of various old magnetic data might be better employed in investigating the secular changes of all the magnetic elements, instead of one only, especially since, as far as is known, the question had heretofore received but scant attention.

In this study the various old magnetic data had to be sorted out and co-ordinated for a large part of the eastern half of the western hemisphere in order to obtain consistent results. A short summary will serve to elucidate the methods used.

The values of declination were obtained by constructing charts for each half century using as a basis old charts such as that of Van Bemmelen, the *Arcano del Mare* and those in Hansteen's *Magnetismus der Erde*, modified and extended by early observations in Canada, such as those of Jacques Cartier, 1534, Master John Davis, 1585, Samuel de Champlain, 1610, Capt. Baffin, 1615, and others.

The change in horizontal force for each half-century interval was found by constructing, from the changes found in the declination, the horizontal magnetic field producing those changes.

<sup>1</sup> *Manuscript received May 6, 1933.*

<sup>2</sup> *Contribution from the Topographical Survey, Department of the Interior, Ottawa, Canada.*

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The values of inclination or dip were obtained by constructing charts for each century using as a basis the old charts of Hansteen, modified and extended by early observations such as those given in the *Magnetismus der Erde*, publications of the United States Coast and Geodetic Survey; and observations of Sir J. H. Lefroy, Sir George Back, Dr. Rae, Sir John Richardson, Thos. Simpson, Esq., Sir John Franklin, Sir E. W. Parry, Sir John Ross, and others.

While a single old original observation of declination or inclination may not be very accurate, average results derived from a considerable number obtained from many sources, such as were used in this investigation, are fairly good. It is conservatively estimated, from a long study of such data, that the maximum error of the old values of declination and inclination here quoted cannot exceed a quarter of one degree. Values quoted for 1850 and later are, of course, quite accurate.

The values of vertical and total force were derived from the values of horizontal force and inclination. The results are shown in Table I.

TABLE I

SECULAR CHANGES OF THE MAGNETIC ELEMENTS, MAJOR'S HILL PARK, OTTAWA  
(Longitude, 75° 41'.8; Latitude, 45° 25'.7)

Date	D	I	H	V	F
1500	7° 00' W.	76° 34'	0.1480	0.6200	0.6374
1550	10 30	76 55	.1400	.6025	.6186
1600	14 09	75 23	.1500	.5750	.5942
1650	16 29	73 07	.1700	.5600	.5852
1700	13 49	73 00	.1804	.5900	.6169
1750	9 50	74 27	.1730	.6220	.6456
1800	6 31	75 48	.1590	.6284	.6482
1850	8 46	76 40	.1450	.6118	.6288
1900	12 26	75 41	.1515	.5936	.6127
1930	14 28	75 31	.1470	.5691	.5878

*D*=declination or variation; *I*=inclination or dip; *H*, *V* and *F*=horizontal, vertical and total force, respectively, expressed in C.G.S. units.

This table shows that for the period of the 430 years considered, all the magnetic elements and the total force have changed with time, in apparent cycles having a period of about 325 years, but that apparently none have suffered any permanent change.

Attempts to express the apparent cycles of secular variations by harmonic analysis, exponential series or other mathematical expressions, have failed. It seems that there are many harmonics composing each secular variation, and that upon the harmonics are superimposed other fluctuations of temporary character or having a shorter period which will have to be eliminated before the true harmonic secular variation can be found. It is well known, by observation, that each station seems to have a different period for its secular variations, which differences are probably caused by temporary fluctuations

of shorter period. However, the reason for the difference in periods among stations is not yet solved. If more tables of secular variations, such as that quoted above, were available, many interesting questions in terrestrial magnetism, including the length of period for secular variations, might be nearer solution.

#### *Note 1*

Magnetic values observed at Major's Hill Park, Ottawa, by the Topographical Survey, Department of the Interior, 1930, were as follows: declination,  $14^{\circ} 28' \text{ W.}$ ; inclination,  $73^{\circ} 31'$ ; total force, 0.5878 c.g.s.

The horizontal and vertical forces for 1930 were computed from the total force and the inclination. The longitude and the latitude of the magnetic station were scaled off a map of one mile to one inch.

#### *Note 2*

In tracing out secular changes in inclination, use was also made of the fact that for stations having a high value of inclination, the inclination varies roughly inversely with the horizontal force.

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## THE FUNGI FOUND IN BUTTER<sup>1</sup>

By G. R. BISBY<sup>2</sup>, M. C. JAMIESON<sup>3</sup> AND M. TIMONIN<sup>4</sup>

### Abstract

Samples of butter from all the creameries in Manitoba were investigated as to mold content. The various species of molds isolated and their relative abundance are listed. A list of fungi recorded as occurring in butter is given. It is pointed out that fungi of various kinds may enter butter, and some of them may develop objectionable colonies when butter is held for some time in transportation or storage. Certain creameries are reported as producing butter practically free from fungi, and suggestions are given regarding methods of lessening the development of molds in butter.

### Introduction

During recent years buttermakers in Manitoba and other parts of Canada have endeavored to produce butter of increasingly high quality. The results achieved have been excellent for the most part, but one rather serious source of trouble arose when butter was shipped to England: molds developed in a number of these shipments. The importance of preventing moldiness in Canadian butter is emphasized by Hood and White (8).

Dairy bacteriologists in Manitoba have determined, for several successive years, the prevalence of molds in representative samples of butter from various creameries, and have aided the buttermakers in their efforts to reduce the numbers of fungi to a minimum. This article presents an analysis of the identity and prevalence of molds found in butter during 1932, and suggests preventive measures. The work was done in conjunction with work on soil fungi (1), since the majority of the fungi found in butter occur also in soil. Many of the species isolated have not been recorded previously in dairy products.

The literature dealing with molds in butter, up to 1929, is summarized by Macy (9), who made extensive experiments to determine the food supply of certain fungi found in butter, and the relation of moisture, temperature, air supply, and salt to mold development. Macy presents a list of the fungi recorded from butter. Grimes, Kennelly and Cummins identified many fungi found in butter (6) and milk (4) in Ireland, and studied their growth on various media. A later paper from Ireland (7) deals with species of *Phoma* found in

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butter. Molds in butter are also being investigated in New Zealand (12) and elsewhere.

The results reported in this paper were obtained from two types of creamery butter made from pasteurized cream. One was the regular creamery output, the other a special product made painstakingly by certain creameries for competition purposes.

The samples of butter from which isolations were made, were taken at the time of grading by staff members of the Provincial and Federal Dairy Branches. Four, three, or two samples per month, depending on the quantity manufactured, were taken from each of the 55 creameries of Manitoba. Due care was taken to avoid external contamination when sampling. A wrapped sterile knife was used to cut each sample of about two ounces, taken from diagonally opposite corners of the parchment-lined boxes of butter. By this method three exterior surfaces were included in each case. The samples were placed at once in sterile screw-top jars, and kept in a refrigerator until analyzed.

Mold and yeast counts were made by the technique of the Committee on Bacteriological Analysis of Dairy Products (3). In this paper, yeast counts are not included under the heading of "molds". The samples were carefully melted to a creamy consistency, and platings made from 1 cc. and 1/10 cc. of butter, using Difco malt agar medium acidified to a pH of 3.5 (14). Incubation was at 25° C. for five days (15). Counts were then made and recorded on a cubic centimetre basis.

Colonies of fungi to be identified were taken from certain plates to test tubes. As far as possible, one plate per month from each creamery was selected for these transfers, and in addition any colonies that appeared to be different were removed from extra plates. Non-acidified Czapek's agar slopes were used except for Mucorales, which were transferred to tubes of Waksman's agar, and *Oospora* and *Mycoderma* which were placed on malt agar slopes. These cultures were incubated at 25° C. until growth was sufficient to permit of a taxonomic study.

### The Number of Molds Found in Butter

During the summer of 1932, 858 samples of butter were studied. Table I lists the samples arranged according to numbers of molds found.

TABLE I  
NUMBERS OF MOLDS FOUND IN SAMPLES OF BUTTER

Number of molds per cc.	Number of samples	Per cent of total
None found	258	30.1
1 to 10	388	45.2
11 to 100	164	19.1
Over 100	48	5.6
Total	858	100.0

Table I shows that nearly one-third of the samples of butter had been made with such care that no contaminating molds developed on the plates. Three-fourths of the samples contained not more than ten molds per cc. of butter.

Table II gives the results obtained in 1932 by a few creameries representative of various degrees of efficiency in controlling mold contamination.

TABLE II

CLASSIFICATION OF CERTAIN CREAMERIES ACCORDING TO NUMBERS OF MOLDS FOUND

Creamery	Samples classified according to numbers of molds per cc. of butter				Total samples tested
	0	1-10	11-100	Over 100	
A	21	1	0	0	22
B	18	4	0	0	22
C	14	4	0	0	18
D	11	7	0	0	18
E	10	6	0	0	16
F	6	6	2	2	16
G	6	11	4	1	22
H	6	6	3	7	22
I	0	5	0	9	14

Creamery A achieved an enviable record during 1932. One colony developed from one sample; all others tested were found to be free from both molds and yeasts. During the years 1930 and 1931 this creamery did even better: no fungi were found in any of the 22 samples analyzed each year. This creamery has demonstrated that butter can be produced commercially without molds or yeasts.

On the basis of mold counts, creameries B and E are good, F and G fair and H and I poor.

Several Manitoba creameries have been able to produce butter relatively free from molds by exercising great care in all stages of the manufacturing process. This accomplishment may be attributed to stimulation of effort resulting from: direct supervision by the Provincial and Federal Dairy Branches; Dairy School instruction; systematic laboratory analyses of butter; the inclusion of mold and yeast counts in the official score card for exhibition butter; and prizes awarded for the lowest counts of fungi during the season.

The value of care in every detail of manufacture is indicated in Table III, obtained by comparing the results achieved by creameries providing special

TABLE III

MOLDS IN SPECIAL AND COMMERCIAL BUTTER

Type of butter	Number of samples	Per cent of samples in classes of molds per cc.			
		0	1-10	11-100	Over 100
Special	146	41.8	47.4	9.5	1.3
Commercial	404	32.4	44.3	17.6	5.7

competitive samples as well as regular commercial butter\*. The competitive samples had significantly fewer fungi.

\*The writers are grateful to Professor N. James, of the Bacteriology Department, for much assistance, and to the staffs of the Provincial and Federal Dairy Branches, for taking the samples.

### The Groups of Fungi Found in Butter

All Phycomycetes isolated belonged to the Mucorales. Only six cultures (1%) were found in this group, whereas 5% of the soil fungi were Mucorales (1). All the five species isolated from butter are known to occur in soil.

Ascomycetes were found twice only: the *Chaetomium* probably developed in nature on manure, the *Valsa* on a twig.

Basidiomycetes might enter the creamery as spores carried in the air, but only one culture was recognized as belonging to this class of fungi.

The Fungi Imperfecti constituted nearly 99% of the isolations. Various *Penicillia* appeared, this genus being represented by 17% of the total number of isolations. Dr. Thom kindly examined cultures of the various species. There seems to be little evidence that certain forms of *Penicillium* should be considered as characteristic of butter; the species most common in butter are common in soil also. It is curious, however, that *P. intricatum* and *P. janthinellum*, found to be two of the commonest soil *Penicillia* in Manitoba, did not appear in butter. The writers are glad to acknowledge Dr. Thom's help with species of *Aspergillus* and *Paecilomyces* also.

*Oospora lactis* (*Oidium lactis*) and *Mycoderma* spp. were exceedingly common in butter. Relatively few of these fungi were transferred from the plates to the 614 test tubes which were studied.

*Alternaria* and *Cladosporium* are two dark-spored genera (Dematiaceae) commonly found in butter, which may be particularly objectionable because of the dark colonies produced.

*Fusaria* occasionally developed in cultures. Dr. W. L. Gordon kindly identified the four species which appeared.

Most of the other Imperfecti are common molds. *Phoma hibernica*, however, constitutes an unsolved mystery.

### List of Species Isolated

The numbers in parentheses following the scientific names of the fungi refer to the numbers of times the species appeared in the isolations studied. These numbers represent roughly the relative frequency of the various species isolated, except that yeasts, which are very common in butter, were not isolated, and *Oospora lactis* and *Mycoderma* spp., which were also very common, were not transferred to test tubes in proportion to their prevalence.

When a remark is included within the parenthesis regarding the occurrence of a fungus in soil or elsewhere, it is to be understood always that soil (see 1) or other strata in Manitoba is meant. Very brief notes are given regarding certain species of fungi.

***Absidia spinosa*** Lendner (1, occasional also in soil).

***Alternaria tenuis*** Nees group (5, also in soil).

*Alternaria* spp. (59, common in soil and on old parts of plants). The dark growth produced by *Alternaria* is particularly objectionable in butter.

*Aspergillus flavipes* (Bain. and Sart.) Thom and Church (3, common in soil).

*Aspergillus flavus* Link (5, also in soil).

*Aspergillus fumigatus* Fres. (4, occurs in soil and on debris).

*Aspergillus niger* van Tieg. (3, not found to be common in soil or on plant parts).

*Aspergillus terreus* Thom (2).

*Aspergillus ustus* (Bain.) Thom. and Church (1, occasional in soil).

*Aspergillus* sp., perhaps *nidulans* group (2). Perithecia with purplish ascospores are produced in culture.

*Basidiomycete*. One culture produces white mycelium with clamp connections, but no spores. One or two other cultures may possibly be Basidiomycetes.

*Botrytis cinerea* Pers. (5, occurs on plants and in the soil).

*Cephalosporium acremonium* Corda (2, also in soil).

*Chaetomium setosum* Wint. (1). Setae first appear as lanceolate bristles, and later dichotomously branched hairs are found. Spores  $6-8 \times 4-5 \mu$ .

*Cladosporium herbarum* (Pers.) Link (22, common on debris and in soil). Some of the forms may represent *C. butyri* Jensen, or possibly other species. *C. herbarum* is recorded (2) as generally present in molded butter.

*Coniosporium arundinis* (Corda) Sacc. (1, found once in soil).

*Coniothyrtum* sp. (4, all from one sample). Spores greenish-brown,  $4-8 \times 3-4 \mu$ .

*Cunninghamella verticillata* Paine (1). This distinctive fungus has oval spores mostly  $10-14 \mu$  long, covered with hair-like projections.

*Diplodia* sp. (1). Spores  $14-16 \times 6 \mu$ , brown, two-celled.

*Fusarium ?bulbigenum* Cke. and Mass. (3).

*Fusarium dimerum* Penz. (3). Spores small, lunar with one or two cells.

*Fusarium moniliforme* Sheld. (4, also in soil).

*Fusarium ?poae* (Peck) Wollenw. (1, found in soil).

*Geotrichum candidum* Link (1, apparently identical with soil isolations).

*Helminthosporium sativum* Pam., King, and Bakke (1, common on cereals and in soil).

**Hymenula affinis** (Faut. and Lam.) Wollenw. (6, also in soil).

**Monilia sitophila** (Mont.) Sacc. (3, occasional in soil).

**Mucor ?circinelloides** van Tiegh. (2). Produces in culture a short turf, darkened with sporangia near the surface of the agar; the sporangiophores are branched, the spores  $5-7 \times 2\frac{1}{2}-3\frac{1}{2} \mu$ .

**Mycoderma** spp. (90 examined). Very common in butter. The cultures superficially resemble those of *Oospora lactis*. Both fungi doubtless indicate that the pasteurized cream had been recontaminated from unsanitary equipment with traces of raw cream or milk.

**Oospora lactis** (Fres.) Lindau (87 examined). Some variation occurs in cultures; the spores, or mycelial segments, are from  $4 \mu$  to  $25 \mu$  or longer; the cultures usually are whitish, but sometimes are pink. Other species of *Oospora* possibly may be included.

**Paecilomyces aurea-cinnamomeum** (Biourge) Thom (1 isolation obtained in 1931).

**Paecilomyces varioti** Bain. (22). This fungus, sometimes known as *Penicillium divaricatum*, is easily recognized by its spreading brown growth bearing penicillate heads with elliptical spores. Although common in butter, it has not yet been found elsewhere in Manitoba.

**Penicillium atramentosum** Thom (1, determined as apparently this species).

**Penicillium aurantio-brunneum** Dierckx (1, occasional in soil).

**Penicillium brevi-compactum** Dierckx (5). Strains show considerable variation.

**Penicillium chrysogenum** Thom (25, plus about 250 colonies from one sample badly contaminated with this species. Also very common in soil).

**Penicillium ?cyclopium** Westl. (2).

**Penicillium expansum** Link series (2).

**Penicillium ?griseum** Sopp. (as *Citromyces*; not *P. griseum* Bonord., 3 cultures).

**Penicillium implicatum** Biourge (1). Dr. Thom considers that this is a new variety characterized by the conspicuous development of transpired drops.

**Penicillium johannioli** Zaleski (2).

**Penicillium lanosum** Westl. (5).

**Penicillium martensii** Biourge (1).

**Penicillium oxalicum** Currie and Thom (2).

***Penicillium purpurogenum*** Stoll (2, occasional in soil).

***Penicillium ?purpurrescens*** Sopp (3, also in soil).

***Penicillium restrictum*** Gilm. and Abb. (1, common in soil).

***Penicillium roqueforti*** Thom (3). Strains of this species are used in making Roquefort cheese.

***Penicillium rugulosum*** Thom (7, common in soil).

***Penicillium sanguineum*** Sopp (1).

***Penicillium spinulosum*** Thom (6, occasional in soil).

***Penicillium terrestre*** Jensen (26, abundant in soil).

***Penicillium viridicatum*** Westl. (1, also in soil).

***Penicillium*** spp. (4 cultures not yet determined).

***Phoma hibernica*** Grimes, O'Connor and Cummins (52). Abundant brownish pycnidia are produced, and masses of flesh-colored spores exude from them. The cultural characters agree with those described (7) for *P. hibernica*, although the spores in the Manitoba isolations are usually somewhat narrower, being  $4-7 \times 2-3 \mu$ . This fungus, common in dairy products in Ireland and Manitoba, has not been found in or on other substrata. It was not encountered in an examination of several thousand fungi isolated from soil in Manitoba.

***Phoma*** spp. (22). At least three species were found, one with spores  $4 \times 1 \mu$ , another with spores  $4-7 \times 3 \mu$ , and a third with spores  $7-11 \times 2-3 \mu$ . These *Phoma* stages no doubt represent contaminations from various plant sources.

***Rhizopus elegans*** Eidam (1, frequent in soil).

***Rhizopus nodosus*** Namysl (1).

***Septoria*** sp. (7 from one sample). Spores curved,  $18-24 \times 1\frac{1}{2}-2 \mu$ , in pycnidia.

***Sporotrichum roseum*** Link (20, also in soil). Spores  $3-6 \times 2-3 \mu$ .

***Stemphylium*** sp. (1).

***Torula*** spp. (3). As a rule yeasts were not isolated. However, some forms resembling molds proved to be *Torula*, possibly *Chromotorula*.

***Trichoderma koningi*** Oud. (14, common in soil).

***Trichoderma lignorum*** (Tode) Harz (16, common in soil and on old wood).

***Valsa*** sp.? (1). Produces stromata with perithecia containing asci with allantoid spores  $8-10 \times 1\frac{1}{2}-2 \mu$ .

**Undetermined fungi** (25.) Fourteen isolations did not produce spores on any of the media tried, and were finally discarded. Eleven other cultures are not yet named, although spores are present.

TABLE IV  
SUMMARY OF FUNGI LISTED ABOVE

Class and genus	Number of entries	Number of isolations	Class and genus	Number of entries	Number of isolations
<b>PHYCOMYCETES</b>			<b>FUNGI IMPERFECTI</b>		
<i>Absidia</i>	1	1	<i>Geotrichum</i>	1	1
<i>Cunninghamella</i>	1	1	<i>Helminthosporium</i>	1	1
<i>Mucor</i>	1	2	<i>Hymenula</i>	1	6
<i>Rhizopus</i>	2	2	<i>Monilia</i>	1	3
			<i>Mycoderma</i>	1	90
<b>ASCOMYCETES</b>			<i>Oospora</i>	1	87
<i>Chaetomium</i>	1	1	<i>Paecilomyces</i>	2	23
<i>Valsa</i>	1	1	<i>Penicillium</i>	22	104
			<i>Phoma</i>	2	74
<b>BASIDIOMYCETE</b>	1	1	<i>Septoria</i>	1	7
			<i>Sporotrichum</i>	1	20
<b>FUNGI IMPERFECTI</b>			<i>Stemphylium</i>	1	1
<i>Alternaria</i>	2	64	<i>Torula</i>	1	3
<i>Aspergillus</i>	7	20	<i>Trichoderma</i>	2	30
<i>Botrytis</i>	1	5			
<i>Cephalosporium</i>	1	2	<b>UNDETERMINED</b>	—	25
<i>Cladosporium</i>	1	22			
<i>Coniosporium</i>	1	1			
<i>Coniothyrium</i>	1	4			
<i>Diplodia</i>	1	1	<b>Totals:</b>		
<i>Fusarium</i>	4	11	29 genera		
				65 entries	614 cultures

As a result of studies made on approximately 75 species of fungi isolated, 54 were specifically determined, 11 were placed in the genus only, and about 10 remain undetermined. Some species are included as doubtful. Nearly all the fungi isolated were saprophytes, although a few, as mentioned below, may have been parasites upon plants. Yeasts, *Oospora* and *Mycoderma*, were the commonest fungi found in butter, but *Penicillium*, *Phoma*, and *Alternaria* are common also.

Table V summarizes the molds recorded as found in butter by previous investigators and in this paper, and gives the probable or possible source of the molds.

It is noteworthy that of the 104 fungi entered below, only 3 are referred to in all three papers cited: these are *Alternaria* spp., *Cladosporium herbarum*, and *Oospora lactis*. *O. lactis* occurs in milk everywhere; the other two fungi are common molds on decaying parts of plants. It is probable that the fungi recorded as found in butter nearly all arise from spores or bits of mycelium from the soil, plants, debris, and manure; they are carried by the air or dust particles and contaminate the cream, equipment or butter. Most of the fungi found in butter produce spores abundantly.

TABLE V  
FUNGI RECORDED IN BUTTER

Fungus	Recorded in			Probable habitat in nature	Fungus	Recorded in			Probable habitat in nature
	A*	B**	C†			A*	B**	C†	
<i>Abdisia spinosa</i>			+	Soil‡	<i>Mucor sylvaticus</i>	+			Soil
<i>Acrostalagmus cinnabarinus</i>		+		Plants††	<i>Mycoderma</i> spp.		+		Milk
<i>Alternaria tenuis</i>			+	Plants	" <i>Oidium varicolor</i> "	+			?
<i>Alternaria</i> spp.	+	+		Plants	<i>Oospora (Oidium) lactis</i>	+	+	+	Milk
<i>Aspergillus flavipes</i>			+	Soil	<i>Oospora ruberrima</i>	+			Debris
<i>Aspergillus flavus</i>	+		+	Plants soil	<i>Paecilomyces aureo-cinnamomeum</i>			+	Debris
<i>Aspergillus fumigatus</i>		+	+	Plants soil	<i>Paecilomyces variotis</i>			+	Debris, soil
<i>Aspergillus glaucus</i>	+	+		Plants	<i>Penicillium atramentosum</i>			+	Cheese ?
<i>Aspergillus luteo-niger</i>		+		Plants	<i>Penicillium aurantio-brunneum</i>			+	Soil
<i>Aspergillus niger</i>	+		+	Plants	<i>Penicillium brevicompactum</i>		+	+	?
<i>Aspergillus oryzae</i>	+			Plants	<i>Penicillium brevicaulis</i>	+			Plants, soil
<i>Aspergillus sydowii</i>		+		Plants	<i>Penicillium chrysogenum</i>	+		+	Soil, debris
<i>Aspergillus terreus</i>		+	+	Soil plants	<i>Penicillium crustaceum</i>	+			?
<i>Aspergillus ustus</i>			+	Soil	<i>Penicillium cyclopsium</i>			+	Plants
<i>Botrytis cinerea</i>		+	+	Plants	<i>Penicillium expansum</i>	+		+	Plants, soil
<i>Cephalosporium acremonium</i>			+	Soil fungi	" <i>Penicillium glaucum</i> "	+			?
<i>Chaetomium botrychoides</i>	+			Manure	<i>Penicillium ?griseum</i>			+	Soil
<i>Chaetomium selosum</i>			+	Manure, debris	<i>Penicillium implicatum</i>			+	?
<i>Cladosporium butyri</i>	+			Milk ?	<i>Penicillium johannisoli</i>		+		Plants
<i>Cladosporium herbarum</i>	+	+	+	Plants soil	<i>Penicillium lanosum</i>			+	Soil?
<i>Coniosporium arundinis</i>		+	+	Hay soil	<i>Penicillium maritensii</i>			+	Plants
<i>Coniothecium</i> sp.	+			Plants	" <i>Penicillium ?olsvaceum</i> "	+			?
<i>Coniokyrium</i> sp.			+	Plants	<i>Penicillium oxalicum</i>			+	Soil, plants
<i>Cunninghamella verticillata</i>			+	Soil	<i>Penicillium purpurogenum</i>			+	Soil
<i>Dematium pullulans</i>	+			Plants	<i>Penicillium ?purpurrescens</i>			+	Soil
<i>Diplodia</i> sp.			+	Plants	<i>Penicillium restrictum</i>			+	Soil
<i>Epicoccum ?heterochroum</i> (2)				?	<i>Penicillium roqueforti</i>	+			Milk?
<i>Epicoccum</i> sp.	+			Plants	<i>Penicillium rugulosum</i>			+	Soil
<i>Eurotium repens</i>	+			Plants	<i>Penicillium sanguineum</i>			+	Soil
<i>Eurotium rubrum</i>	+			Plants	<i>Penicillium sartoryi</i>		+		?
<i>Fusarium ?bulbigenum</i>			+	Plants	<i>Penicillium spinulosum</i>		+	+	Soil, plants
<i>Fusarium dimerum</i>			+	Plants	<i>Penicillium terrestre</i>			+	Soil
<i>Fusarium moniliforme</i>			+	Plants soil	<i>Penicillium viticatum</i>			+	Plants
<i>Fusarium ?poae</i>			+	Plants soil	<i>Phoma hibernica</i> (see 7)		+	+	?
<i>Fusarium reticulatum</i>		+		Plants	<i>Phoma destructiva</i> (see 7)		+		Plants
<i>Geotrichum candidum</i>			+	Plants	<i>Rhizopus 'arizus'</i>	+			
<i>Gliocladium</i> spp.		+		Plants soil	<i>Rhizopus elegans</i>			+	Soil
<i>Graphium penicillioideis</i>		+		Wood	<i>Rhizopus nodosus</i>			+	Soil
<i>Helminthosporium sativum</i>			+	Cereals	<i>Septoria</i> sp.			+	Plants
<i>Hymenula affinis</i>			+	Soil	<i>Sporotrichum carnis</i>		+		?
" <i>Monilia alba</i> "	+			Plants	<i>Sporotrichum roseum</i>			+	Soil
<i>Monilia candida</i>	+			Wood	<i>Stemphylium butyri</i>	+			Milk?
<i>Monilia sitophila</i>			+	Debris soil	<i>Stemphylium ericoclonum</i>		+		Plants
" <i>Monilia rosea</i> "	+			Debris	<i>Stemphylium</i> spp.		+	+	Plants
<i>Mucor ? circinellodes</i>			+	Soil plants	<i>Stysanus microsporus</i>		+		Plants
<i>Mucor corymbifer</i>		+		Animals	<i>Torula</i> sp.		+	+	?
<i>Mucor hiemalis</i>	+			Soil	<i>Trichoderma koningsi</i>			+	Soil
<i>Mucor mucedo</i>	+			Manure, soil	<i>Trichoderma lignorum</i>			+	Soil, wood
<i>Mucor petriularis</i>	+			?	<i>Trichosporium collae</i>	+			Debris
<i>Mucor racemosus</i>	+			Manure, soil	<i>Trichothecium roseum</i>		+	+	Plants
<i>Mucor spinosus</i>	+			Dung, debris	<i>Valsa</i> sp.			+	Plants
					<i>Verticillium</i> sp.	+			

\*A—Macy's summary (9). \*\*B—Grimes et al (6). †C—This paper. ‡"Soil" fungi of course live for the most part on the remains of plants. ††"Plants" refers usually to old decaying plant parts.



It may be noted further that 32 or approximately half of the 65 fungi identified in Manitoba butter were isolated also from soil there (1). This indicates that soil, perhaps dust more particularly, is an important source of the fungi which find their way into cream or butter. During the dry summers such as have prevailed in recent years in western Canada, the total exclusion of dust particles requires extreme care.

Most of the fungi found in butter, including many of those occurring also in soil, develop more or less abundantly upon plants or plant parts, usually as saprophytes. A few species, such as those of *Fusarium*, *Helminthosporium*, *Diplodia*, *Septoria* and certain *Phomas*, are known to occur as parasites on plants. The spores of these fungi must be common in the air. A few fungi such as *Trichoderma lignorum* may develop upon wood in groves or the wood yard. *Oospora*, *Mycoderma* and yeasts multiply profusely in infected cream or milk.

Pasteurization of cream has been shown to be effective in killing the fungi present. The utmost vigilance is required to protect the product subsequent to pasteurization.

### Inoculations upon Butter

Macy (9) and others have studied the growth of molds on various media, including sterile butter. The writers made a few tests of the more common fungi by inoculating them directly upon slices of salted and unsalted butter in Petri dishes. A good grade of creamery butter was used, which however already contained a few mold spores. When these slices of butter were inoculated and left for one to three weeks, scarcely any visible growth appeared upon them. Butter is rather low in moisture, and some evaporation from the Petri dishes occurs in the dry atmosphere of the laboratory. Accordingly 5 cc. of sterile water was added to each of a set of plates containing inoculated salted and unsalted butter. After a month, a small amount of growth from inoculations with *Alternaria* and *Oospora* only, appeared on the salted butter. Every plate of unsalted butter, however, showed conspicuous development of molds, to a considerable extent consisting of *Penicillia* and *Trichoderma* arising from contamination of the butter previous to inoculation. These few preliminary experiments suggest that low moisture content and the addition of salt are of value in restricting growth of molds, as found by Macy (10) and Voitkevich (13).

### Discussion

It is exceedingly difficult, but not impossible, to exclude fungi from butter. Macy and Richie (11) and Grimes (5) found that the flavor and quality of fresh butter showed no correlation with the micro-organisms present (in a more or less dormant condition) in the samples. Macy and Richie (11) found, however, a tendency toward slightly better keeping quality when the yeast and mold counts were low. Certainly every effort should be made to minimize contamination of butter by foreign material; sanitary precautions automatically reduce the numbers of fungi in butter. Butter to

be shipped overseas, or otherwise held long in storage, should have the lowest possible count of fungi to start with, and should be kept at low temperature and low humidity. The addition of salt to butter lessens the development of molds.

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## A PARASITOLOGICAL SURVEY OF THE GENUS *CITELLUS* IN MANITOBA<sup>1</sup>

By J. A. McLEOD<sup>2</sup>

### Abstract

A parasitological survey of 236 gophers (*Citellus* spp.) in the province of Manitoba yielded five previously recorded ectoparasites, four species of Nematodes, three of which are regarded as new to science, an Acanthocephalan and two previously unrecorded species of Cestodes. No Trematodes or intracellular Protozoa were found.

The importance of the Arthropod parasites as transmitters of disease, the pathogenicity of the enteric parasites and the correlation between the incidence of infestation and host abundance, are discussed.

Three species of *Citellus* commonly known as gophers are responsible for an immense annual loss to the farming industry of western Canada both in the destruction of growing crops and in the impeding of land cultivation by burrowing (6, 12, 14). There is also the possibility of such common rodents serving as alternate hosts, or as the direct carriers of organisms pathogenic to higher animals as do a number of closely related forms in other parts of the world (15, 22, 27).

It is well known that ground rodents are particularly susceptible to cyclic fluctuations of population density (3, 7, 8). Factors inducing such fluctuations are obscurely known but climatic extremes undoubtedly play a part. Overcrowding reduces the potential fertility. Bacterial and helminth parasitism may be pathogenic. Endemic helminth infestation may become epidemic under conditions of overcrowding or adverse climatic conditions with increased mortality and local fluctuations of population density as shown by Boughton (3).

Campaigns for the artificial control of these rodents by trapping, poisoning, burrow fumigation, etc., carried out at considerable expense in the past would appear to be unwarranted if epidemic disease regularly occurs when the population has reached a certain density. The presence of epidemic mortality among gophers is less easily detected than among non-burrowing forms and a continuous record of gopher abundance over the whole of Manitoba is not available. Such evidence as the writer has been able to collect from farmers and naturalists suggests strongly that there exist peak years of abundance followed by a sharp decline in population density. The peak years are not synchronous over the entire area but in the localities studied the maxima of abundance appear to be as follows:

<i>C. tridecemlineatus</i> .	1897-99	1912	1917	1923	1927	1932
<i>C. franklini</i> .		1912	1917	1923	1927	1932
<i>C. richardsoni</i> .		1912	1917	1923	1927	1932

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Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada. This paper was constructed from a thesis presented to the Committee on Postgraduate Studies, University of Manitoba, April, 1933, in part fulfilment of the requirements for the degree of M.Sc.

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The aim of the investigation reported below therefore has been that of surveying the parasitic fauna of the species of gophers present in Manitoba, with a view to ascertaining which members of that fauna have the potentiality of gopher destruction and which have the potentiality of spreading from gophers to man and higher animals in the capacity of disease vectors.

The survey included three species of *Citellus*. *C. franklini* and *C. tridecemlineatus* are indigenous forms representing 2 and 8% of the gopher population respectively, while *C. richardsoni* is an immigrant which came from the southwest about 1900 and represents about 90% of the gopher population (23, pp. 372-416). The report is based on the examination of 236 individuals from the southern half of Manitoba carried out during the period May to October, 1932, a year which appears to be a peak year of gopher abundance.

### Methods

The animals examined during the survey were obtained from as many scattered points as possible for the purpose of comparing the incidence of each of the parasites in the different localities. As the area covered was approximately 80,000 square miles it was impossible to examine material from the different localities at regular intervals in an effort to determine the change of infestation with seasonal change and increase in the age of the host. Most of the animals were obtained alive, were etherized in the laboratory, the fur combed for ectoparasites, and a routine post-mortem examination performed.

The helminthological technique used was the standard technique recommended by other authors (26). Intestinal helminths were obtained by a decantation method, were washed in warm water and fixed in 70% alcohol at 75° C. (a method found satisfactory both for nematodes and cestodes) followed by preservation in 70% alcohol plus glycerol. Nematode preparations were made by clearing in glycerol-alcohol at room temperature and mounting in glycerol jelly. Cestode material was stained with Delafield's hematoxylin and cleared in beechwood creosote. This clearing agent was found most satisfactory also for the ectoparasitic fauna.

### Taxonomy

The survey yielded five ectoparasites and seven endoparasites, which may be listed briefly as follows:

#### ARTHROPODA

Ixodidae.	<i>Dermacentor venustus</i>
Dermanyssidae.	<i>Liponyssus occidentalis</i>
	<i>Liponyssus montanus</i>
Pulicidae.	<i>Ceratophyllus bruneri</i>
Haematopinidae.	<i>Linognathoides montanus</i>

#### CESTODA

Dilepididae.	<i>Prochoanotaenia spermophili</i> , n. sp.
Hymenolepididae.	<i>Weinlandia citelli</i> , n. sp.

## NEMATODA

Strongylidae.

*Warrenius bifurcatus*

Spiruridae.

*Rictularia citelli*, n. sp.*Spirura infundibuliformis*, n. sp.*Physaloptera spinicauda*, n. sp.

## ACANTHOCEPHALA

Moniliformidae.

*Moniliformis spiradentatis*, n. sp.

The absence of Trematoda and of larval Cestoda is noteworthy, but it may be noted that Boughton (3) found no Trematoda and only two larval Cestoda.

All five species of ectoparasites were common to the three species of *Citellus* examined. Of the helminths, some were restricted to one *Citellus* species, others to two, and one occurred in all three host species as shown in Table I. The helminths were all found in the stomach or duodenum—the caecum, large intestine, liver, lungs and muscles being free from these parasites. About 40% of the gophers examined showed lesions of the stomach or intestine wall, but although a considerable number of such lesions were sectioned and examined no intracellular Protozoan was found. Several specimens showed splenic enlargement but time did not permit of the preparation and examination of blood smears of such individuals, and the writer was unaware at that time that a trypanosome had been recorded from *Citellus richardsoni*.

Table I is a record of the extent of infestation for each species of parasite for both young and adult of all three species of host. The young of *C. tridecemlineatus* and *C. franklini* were found to be uninfested up to the age of eight

TABLE I

THE RELATIVE INFESTATION OF THE THREE HOST SPECIES AND THE NUMBER OF PARASITES PER HOST

Parasite	Number examined	Per cent infested	Mean number of worms	Maximum number of worms
<i>C. tridecemlineatus</i>				
<i>Rictularia citelli</i>	71	21.1	4	8
<i>Spirura infundibuliformis</i>	71	19.4	18	48
<i>Physaloptera spinicauda</i>	71	15.4	2	4
<i>Moniliformis spiradentatis</i>	71	8.4	7	19
<i>Weinlandia citelli</i>	71	14	5	15
<i>Prochoanotaenia spermophili</i>	71	2.8	1	1
<i>C. franklini</i>				
<i>Rictularia citelli</i>	11	18.1	3	4
<i>Physaloptera spinicauda</i>	11	18.1	4	7
<i>Weinlandia citelli</i>	11	9	17	17
<i>C. richardsoni</i>				
<i>Warrenius bifurcatus</i>	154	62.0	24	83
<i>Spirura infundibuliformis</i>	154	0.6	2	3
<i>Weinlandia citelli</i>	154	2.9	8	20
<i>Prochoanotaenia spermophili</i>	154	1.9	8	17

weeks but those of *C. richardsoni* were heavily infested, in some cases at an early age. Females showed a slightly heavier infestation than males but this was not great enough to be of any particular significance. The mean and maximum number of parasites per host is of considerable importance however and will be discussed later.

### Descriptions

*Dermacentor venustus* Banks, 1912

(*Dermacentor andersoni* Stiles, 1910)

Typical Ixodid ticks of medium-large size measuring up to 6 mm. in length in the adult.

*Male.* Has a well-developed scutum covering the entire dorsal surface of the body, which is chestnut brown with sparsely scattered white spots and unequal punctations. Scutum bears 13 festoons on the posterior margin. Mouth parts project forward beyond the anterior margin so as to be visible from the dorsal side. Eyes rather flat and at the sides, level with the second legs. The palps are much longer than broad and the second segment is without a retrograde spur. The spiracle is situated at the side of the body behind leg IV and the posterolateral extension of the peritreme is well developed.

*Female.* Scutum small and pale, being only slightly longer than broad and having the posterior border a little sinuous. The eyes, as in the male, are located at the sides, about the middle of the length of the scutum opposite the second pair of legs.

The species *venustus* is distinguished from the closely related species *occidentalis* and *albipictus* by the shape of the spiracle and the well-developed posterolateral extension of the peritreme.

*Localities.* Montana and Wyoming, U.S.A., Manitoba and British Columbia, Canada.

*Ceratophyllus bruneri* Baker, 1895

(*Ceratophyllus* Curtis, 1832)

*Specific diagnosis.* Siphonaptera of medium size with the three thoracic segments not strongly constricted and their epiphyses extending over but one abdominal segment. Abdominal tergites with two transverse rows of bristles. Pronotal comb with 18 ctenidia, genal comb absent and gena without recurved process. Head bluntly rounded anteriorly. Eyes present, genal row with one bristle. Labial palps with four pseudojoints and reach to about the distal end of the femora. Maxillary palpi shorter than the anterior coxa. Legs slender.

*Female.* Third joint of the antenna with nine pseudojoints distinct on the posterior side, but indistinct on the anterior. Second joint of the antenna with a row of fine hairs which extend almost to the distal end of joint III. Gena without recurved process but style present. Three antipygidial bristles present.

**Male.** Body slightly smaller than female and with the posterior end curved dorsally. Third joint of antenna distinctly divided into nine pseudojoints. Gena without recurved process, style absent. Three antipygidial bristles, claspers short, smooth on the ventral margin, and bristles are in a small group of five near the upper end.

**Host and locality.** *Citellus* sp. Montana; *C. tridecemlineatus*, *C. franklini* and *C. richardsoni*, Manitoba.

*Liponyssus occidentalis* Ewing, 1923

(*Liponyssus* Kolenati, 1859)

**Female.** Small with large dorsal shield. Palpi moderate, chelicera stout. Dorsal shield extending across the body at the shoulders, lateral margins behind the shoulders convex. Peritreme long and very sinuous, reaching to the anterior coxa. Sternal plate about three times as broad as long, barely reaching to the third coxa, and with the anterior margin strongly arched. Anterior setae situated on the anterior margin; middle setae situated on a line between the anterior and posterior setae, the latter being almost at the tip of the posterior angles. Anal plate egg-shaped in outline; anus small, almost circular with a uniform rim and situated in front of the middle transverse line; paired setae situated near the level of the anterior margin of the anus. Posterior setae situated more than their length behind the anus; caudal area forming a lobe-like projection of the anal plate. Legs moderate. Body length 0.61 mm., width about 0.31 mm.

**Male.** Unknown.

**Hosts.** *C. richardsoni* and *C. tridecemlineatus*.

**Locality.** Montana and Manitoba.

*Liponyssus montanus* Ewing, 1923

**Female.** Large, the body length being about 1.02 mm. and the width about 0.60 mm. Palpi large; chelicera shear-like but the hooked tips of both arms are rather blunt. Dorsal shield medium, lateral margins behind the shoulders very slightly convex. Peritreme long and sinuous and extending to opposite coxa I. Sternal plate with posterior corners broadly rounded and not extended. Anal plate very large, broadly rounded in front and somewhat truncate behind; anus subcircular with a uniform rim, and situated almost centrally; paired setae situated far forward, being at the level of the anterior margin of the anus; median seta situated about its length behind the anus; caudal area crescentic, scobiate. Legs long, anterior pair longer than the second pair and about equal to the third pair. Last pair reaching to about the tip of the abdomen.

**Male.** Unknown.

**Host.** *C. richardsoni* and *C. tridecemlineatus*.

**Locality.** Montana and Manitoba.

*Linognathoides montanus* Osborn, 1912

(Linognathoides Cummings, 1914)

**Specific diagnosis.** First pair of legs smaller than either the second or third pair. Abdominal pleural plates rudimentary. Six pairs of abdominal spiracles present opening on the flat body surface. Abdomen clothed with normal setae with never more than a single transverse row on a typical segment. Antennae five segmented, the second being the longest and the last two being distinct. Temples slightly swollen but without posterolateral angles.

**Male.** Body about 0.84 mm. long and about 0.4 mm. in greatest body width. Head about the size of the thorax; temples swollen; clypeal region pointed, forehead knob-like. Legs stout with well-developed single claws on the tarsi of the first pair. Posterior end broadly rounded. Color, light brown.

**Female.** Body length about 1.15 mm. by about 0.4 mm. in greatest body width. Head antennae and legs similar in size and shape to those of the male. Abdomen large and rectangular, ending bluntly. Body color, dark brown.

**Host.** Species of *Citellus*.

**Locality.** Practically all over the North American continent.

*Warrenius bifurcatus* Hall, 1916

**Generic diagnosis.** Head simple, no lips evident. Bursa is deeply incised dorsally to form two large lateral lobes and a small dorsal lobe. The dorsal, lateral and ventral ray systems are well defined and separated from one another, the rays of each system being more closely related to one another than to the rays of the other systems. The dorsal lobe is supported by the dorsal ray which branches dichotomously. The spicules are well developed, uteri divergent, vulva in the posterior half of the body, ovijector well developed.

**Specific diagnosis.** The worms are whitish in color after fixation but the intestines are red in living specimens. Cuticle is finely striated transversely and presents also about 24 longitudinal striae running the full length of the body. The nerve ring is situated about one-third of the way along the esophagus from the anterior end of the body. The esophagus is dilated posteriorly and separated from the mesenteron by a constriction. The excretory pore is about two-thirds of the way along the esophagus.

**Male.** Length 7–12 mm. Greatest body width 215  $\mu$ . Diameter of head exclusive of cuticular inflation about 36  $\mu$ . Length of the esophagus about 600  $\mu$ . Right spicule is bifurcated in a horizontal plane, left in a vertical. Right lobe of the bursa is about one and one-half times as long as the left.

**Female.** Length 15–18 mm. Greatest body width 315–335  $\mu$ . Diameter of head exclusive of cuticular inflation about 48  $\mu$ . Length of esophagus about 750  $\mu$ . Distance of the anus from the posterior end ranges from 88 to 120  $\mu$ . The vulva is a transverse crescentic slit about 2.6 mm. from the posterior end which bears a minute curved cuticular spine.



*Eggs.* Very numerous in gravid females; 12–16 segmented and enclosed in a thin shell.

*Host.* *C. richardsoni*, in stomach and duodenum.

*Locality.* Manitoba and Saskatchewan, Canada.

This species has been fully described by Sleggs (24).

*Rictularia citelli* n. sp.

(Figs. 1, 2, 3, 4)

*Specific diagnosis.* Buccal capsule well developed and narrow with its aperture more or less distinctly dorsal and with its base armed with teeth and spines. Along practically the entire ventral surface on each side there is a row of cuticular spines. Vulva anterior, near the posterior end of the esophagus. Oviparous, the eggs containing well-developed embryos when oviposited.

*Female.* Rather stout worms measuring from 30 to 60 mm. in length; of a pinkish color when alive but turning almost white on fixation. The following body measurements were found to be constant for gravid females 44 mm. in length. The head including cuticle is  $235\ \mu$  in diameter and the body increases in thickness gradually until at a point one-fifth of its length from the posterior end it is  $910\ \mu$  in diameter. This diameter is held or only slightly reduced to a point a short distance from the caudal end where attenuation takes place sharply, the body ending in a sharp point. Cuticle is about  $12\ \mu$  thick in the head region and is definitely annulated along the neck. There is a pair of stout lateral cervical papillae about  $630\ \mu$  from the anterior end. The first cuticular spine in each row is situated posteriorly and slightly ventrally to the mouth, there being 28 spines in each row from the head region to the vulva. Mouth is reniform in shape and is bounded by two subequal lips. The dorsal lip bears a short conical tooth on each side of its apex while the ventral one is smooth, semicircular and forms the helmet-like anterior termination of the body. The esophagus is simple, slightly dilated in the middle region and ends posteriorly in a hemispherical projection into the intestine. The vulva is a short transverse slit near the posterior end of the esophagus and is bounded by prominent lips formed of cuticle overlying finger-like projections of the body wall. The body is slightly expanded on each side of the vulva. The vagina is long and bifurcates posteriorly to form two convergent uteri which terminate in filiform ovaries.

*Eggs.* The eggs are  $47\text{--}52\ \mu$  in length by  $34\text{--}38\ \mu$  in width, very numerous, elliptical in shape with thick shells and each contains a coiled larva.

*Male.* Like many of the species of this genus, the males of *Rictularia citelli* are rare, there being only a single male found in the 50 specimens examined. This specimen, measuring 14 mm. in length, resembled the female in body shape, presence of cervical papillae, mouth structures and cuticular annulations. There were 28 combs in each row from the head region to the transition

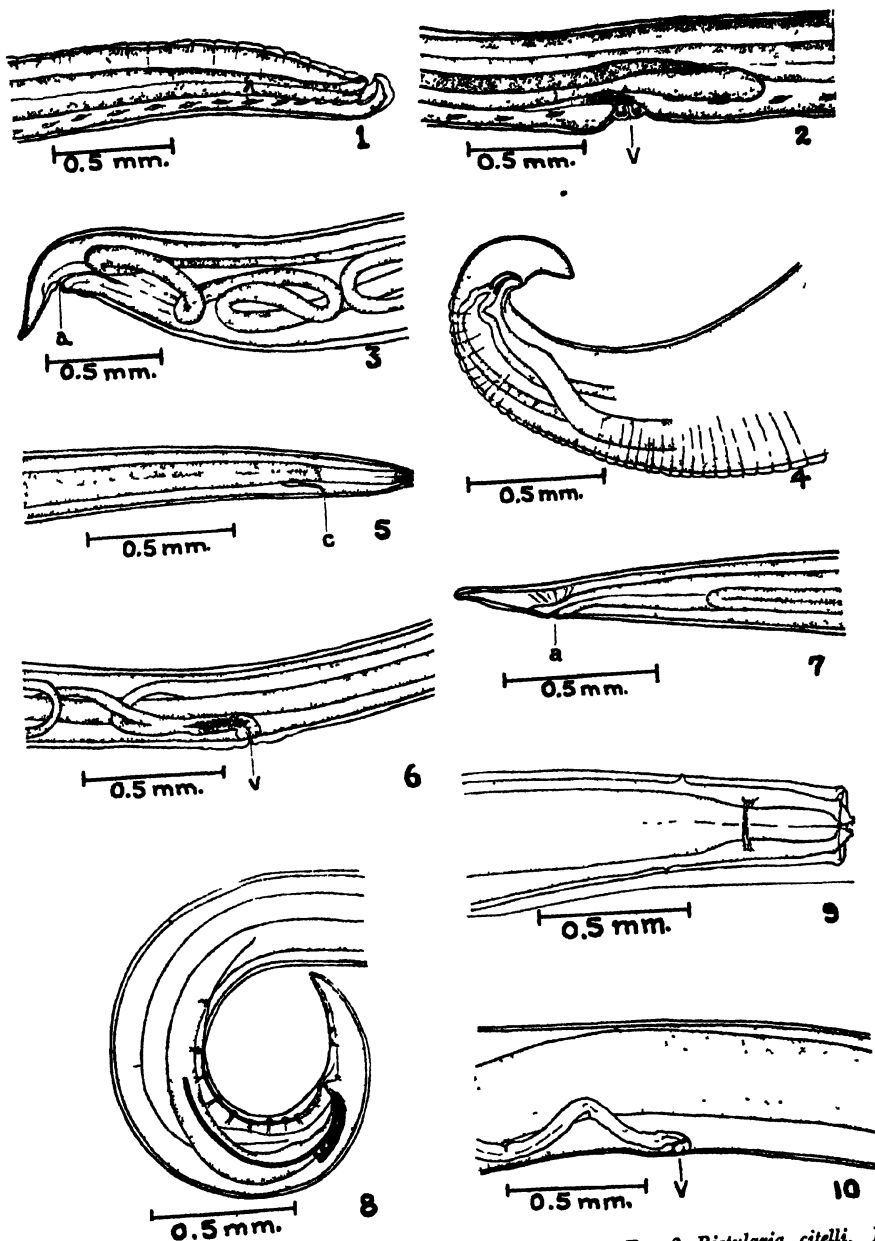


FIG. 1. *Rictularia citelli*. Anterior extremity, lateral view. FIG. 2. *Rictularia citelli*. Body in region of vulva. V, vulva. FIG. 3. *Rictularia citelli*. Female, posterior extremity, lateral view. a, anus. FIG. 4. *Rictularia citelli*. Male, posterior extremity, lateral view. FIG. 5. *Spirura infundibuliformis*. Anterior extremity, lateral view. C, cervical pore. FIG. 6. *Spirura infundibuliformis*. Body in region of vulva. V, vulva. FIG. 7. *Spirura infundibuliformis*. Male, posterior extremity, lateral view. a, anus. FIG. 8. *Spirura infundibuliformis*. Male, anterior extremity, lateral view. FIG. 9. *Physaloptera spinicauda*. Anterior extremity, dorsal view. FIG. 10. *Physaloptera spinicauda*. Body in region of vulva. V, vulva.

point, posterior to which the rows of combs extended almost to the caudal end. The posterior end of the body is obtusely conical, ending in a blunt point and is sharply curved ventrally. The anus is situated on a slight elevation  $227\ \mu$  from the end of the tail. The spicules are equal, small and curved, measuring  $117\ \mu$  in length. A gubernaculum is absent.

The possession of two lips and a definite buccal cavity along with a short muscular and a long glandular region of the esophagus, simple intestine and vulva opening in the midbody region, places this nematode in the superfamily Spiruroidea. The absence of projecting processes on the head and the presence of two longitudinal rows of spines on the ventral surface identifies it as a member of the genus *Rictularia*. The shape and position of the cervical papillae and the number of cuticular spines between the head region and the transition point serve to distinguish this from other species and is considered sufficient grounds for the creation of a new species.

*Type host.* *C. tridecemlineatus* and *C. franklini* (in stomach and duodenum).

*Type locality.* Manitoba, Canada.

*Spirura infundibuliformis* n. sp.

(Figs. 5, 6, 7, 8)

*Specific diagnosis.* Posterior portion of the body decidedly thicker than the anterior portion. Cuticle densely striated transversely. At a distance one-seventh to one-twelfth of the total body length from the anterior end is a prominent cuticular boss or struma. The anterior end is bluntly rounded. Mouth with two inconspicuous lips each bearing three papillae and surrounded by chitinous projections of the vestibule. Vestibule well marked, wide and cylindrical when seen laterally. Esophagus narrow and cylindrical, one-sixth as long as the body.

*Male.* Length 28 to 34 mm. with a gradual increase in body width from the narrow head to a point just anterior to the tail. Body widths for specimens measuring 31 mm. in length are as follows:—head  $79\ \mu$ , mid-cervical region  $210\ \mu$ , posterior to boss  $253\ \mu$ , and posterior region  $367\ \mu$ . The mouth width is  $43\ \mu$  and the length of the vestibule  $87\ \mu$ . Cervical pore opens  $402\ \mu$  from the anterior end and the boss is situated 2.4 mm. from the anterior end. The esophagus is narrow, about one-sixth as long as the body and is divided into an anterior muscular portion reaching posterior to the cervical pore, and a long glandular posterior portion. The anterior end of the body is bent ventrally at an angle of  $45^\circ$  just posterior to the boss. The caudal end is sharply coiled ventrally. Two curved very unequal spicules are present, the right being  $770\ \mu$  in length while the left is  $297\ \mu$ . A gubernaculum is present. The caudal end bears two long narrow alae which meet behind at the tip of the tail but do not meet on the ventral side anterior to the anus. These alae are supported by twelve pairs of pedunculated pre-anal papillae and six pairs of short post-anal papillae, the last two pairs of which are close to the posterior extremity.

*Female.* Length 20–41 mm., and decidedly thicker near the posterior end. The diameters of specimens 40 mm. long are as follows:—head 96  $\mu$ , anterior end of esophagus 175  $\mu$ , behind struma 437  $\mu$ , mid-body and posterior regions 542  $\mu$ . The esophagus is one-sixth as long as the body and the boss is situated 2.17 mm. from the anterior end. The posterior end of the body is straight and conical with an obtuse termination. The anus opens 420  $\mu$  from the posterior end. The vulva is situated on the ventral side about five-eighths of the body length from the anterior end and is a prominent transverse slit with conspicuous lips, the cuticle being slightly thickened for a distance on each side of the aperture. There is a short vagina connecting with an infundibuliform ovijector which has a chitinous lining inside the muscular wall. This chitinous lining is thrown up into folds forming oblique valves on the wall with their free ends directed toward the vagina. The short common trunk of the uterus divides to form two divergent uteri.

*Eggs.* Roundly elliptical in shape and have thick smooth shells. They are very numerous in gravid females and measure from 36 to 39  $\mu$  in length by 27 to 30  $\mu$  in breadth.

The identity of this nematode as a member of the superfamily Spiruroidea is established by the possession of two indefinite simple lips, definite vestibule and long esophagus followed by a simple intestine, and the opening of the vulva near the middle of the body. The presence of a cuticular boss and a definite nerve ring together with well-developed caudal alae in the male supported by costiform papillae places it in the genus *Spirura*. This species resembles to a great extent the species *Spirura talpae* Gmelin, 1790. The females differ in body size, the number and size of the eggs produced and in the posterior body termination. This species terminates in an obtuse cone, the concave surface posterior to the anus, so pronounced in the species *Spirura talpae*, being absent. Apart from body size the males differ only in the number of papillae supporting the caudal alae. This species has twelve pairs of pre-anal and six pairs of post-anal papillae, while the species *talpae* has four pairs of pre-anal and five pairs of post-anal papillae (13).

In the opinion of the writer the nematode described represents a hitherto unrecorded species and the specific name *infundibuliformis* is suggested.

*Type host.* *C. tridecemlineatus* and *C. richardsoni* (in stomach and duodenum).

*Type locality.* Manitoba, Canada.

*Physaloptera spinicauda*, n. sp.

(Figs. 9, 10, 11, 12)

*Specific diagnosis.* Large relatively thick worms with two large simple triangular lateral lips each armed with two teeth and bearing a papilla at its apex. Cuticle may be reflected over the head to form a cephalic collarete or may be retracted and thrown into folds in the anterior cervical region.

Cervical papillae posterior to the nerve ring; buccal cavity is short and a definite vestibule is absent. Esophagus divided into a short muscular anterior part and a long glandular posterior part. Cuticle densely striated transversely.

*Male.* Length from 15 to 20 mm., the body being slightly attenuated at the anterior end. Head round, continuous with the body and with two triangular lateral lips each of which terminates in a short papilla. Each lip bears two short teeth on its inner surface near the base of the papilla. In specimens 18 mm. in length the cuticle was thrown up so as to form a cephalic collarete 52  $\mu$  in length and 280  $\mu$  in diameter. The head was 210  $\mu$  wide with a shallow buccal cavity but a vestibule was absent. Two lateral cervical papillae are present about 700  $\mu$  from the anterior end, the left being slightly posterior to the other. The esophagus is broad and about one-fifth as long as the body, the anterior one-sixth being muscular and the remainder being glandular. A distinct nerve ring is present about 437  $\mu$  from the anterior end. The body is cylindrical and of uniform thickness being about 612  $\mu$ . The caudal end is conical and flattened on the ventral side, ending in a blunt point and is curved ventrally. The large caudal alae which meet anteriorly are supported by four long costiform papillae, two pre-anal and two post-anal. A single sessile median papilla is present about 245  $\mu$  from the posterior body termination. A gubernaculum is absent and the two sub-equal spicules measure 437  $\mu$  and 700  $\mu$ . The anus is situated on an elevation 875  $\mu$  from the posterior end and the curved ventral surface of the caudal region bears numerous longitudinal rows of very fine cuticular projections.

*Female.* Length from 18 to 50 mm. Generally resemble the male with regard to body shape and head structures. Cuticle is very dense and transversely striated. The vulva is a very small inconspicuous circular opening at the end of the anterior one-third of the body. There is a narrow tubular vagina of considerable length with a sphincter muscle at its outer end. The two divergent uteri lie as much-coiled tubes posterior to the vulva in the non-gravid female but in the gravid female the anterior uterus reaches to within a short distance of the esophagus. The tail is acute and ends bluntly, being flattened dorsoventrally. The anus is situated on a prominent elevation and the intestine is large.

*Eggs.* Roundly elliptical, measuring 41  $\mu$  to 44  $\mu$  in length by 28  $\mu$  to 32  $\mu$  in breadth. They are thick shelled and very numerous.

The presence of two large triangular lateral lips and a cephalic collarete and absence of a vestibule, together with large caudal alae supported by long costiform papillae in the males of this species, is considered sufficient reason for placing it in the genus *Physaloptera* of the superfamily Spiruroidea. It differs however from *P. citelli*, the only recorded species from *Citellus*, in general body size and in the possession of only two head papillae. This is considered sufficient grounds for the creation of a new species for which the name *spinicauda* is suggested.

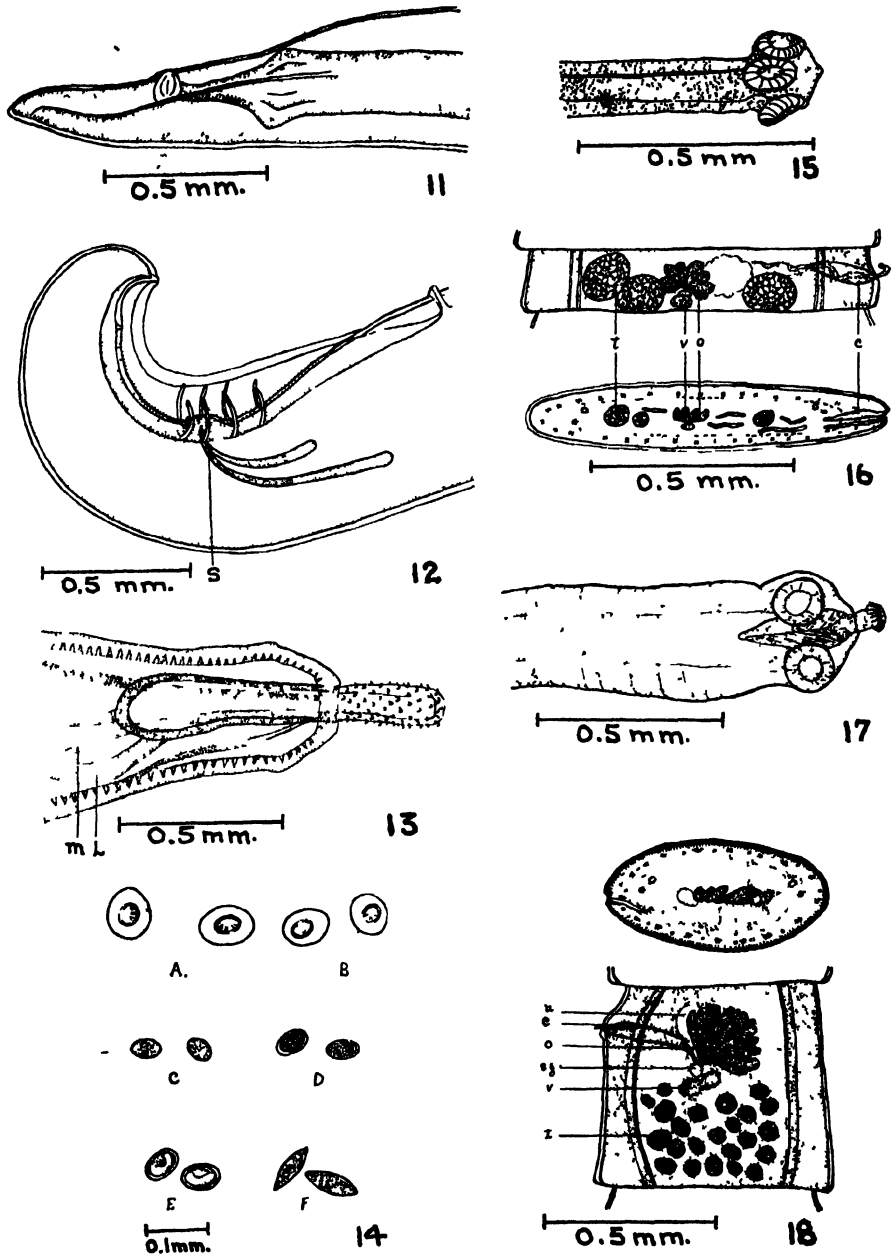


FIG. 11. *Physaloptera spinicauda*. Female, posterior extremity, lateral view. FIG. 12. *Physaloptera spinicauda*. Male, posterior extremity, lateral view. S, spicules. FIG. 13. *Moniliformis spiradentatis*. Anterior extremity, dorsal view. m, muscles; L, limnsoci. FIG. 14. Eggs. A, *Weinlandia*; B, *Prochoanotaenia spermophila*; C, *Physaloptera spinicauda*; D, *Spirura infundibuliformis*; E, *Rictularia citelli*; F, *Moniliformis spiradentatis*. FIG. 15. *Weinlandia citelli*. Scolex and neck, ventro-lateral view. FIG. 16. *Weinlandia citelli*. Proglottis. t, testes; v, vitellarium; o, ovary; c, cirrus sac. FIG. 17. *Prochoanotaenia spermophila*. Scolex and neck, dorsal view. FIG. 18. *Prochoanotaenia spermophila*. Proglottis. u, uterus; c, cirrus sac; o, ovary; sg, shell gland; v, vitellarium; t, testes.

*Type host.* *C. tridecemlineatus* and *C. franklini* (in stomach and duodenum).

*Type locality.* Manitoba, Canada.

*Moniliformis spiradentatis* n. sp.

(Fig. 13)

*Specific diagnosis.* Echinorhynchidea of medium to large size. Body without spines and is divided into a large number of pseudo-segments. Neck absent, proboscis well developed subcylindrical and armed with numerous rows of hooks which are small and have only a single posteriorly directed root. Limnisci filiform, with numerous nuclei. Testes ellipsoidal and situated quite posteriorly; prostatic glands eight, compressed and almost spherical.

These worms are very variable in size even from a single host, the males ranging in length from 35 to 110 mm., while the females range from 45 to 190 mm. The body widths also vary greatly being from 0.73 mm. in the smallest males and females to 1.2 mm. and 2 mm. respectively for the larger specimens. In fully developed worms the body is somewhat flattened and except at the two extremities is marked out into a large number of pseudo-segments; the posterior end is considerably broader than the anterior in large specimens. The proboscis is relatively short, subcylindrical and with a broadly rounded end, the length being 0.40 to 0.42 mm. with a greatest breadth of about 0.16 mm. A short portion of the proximal end of the proboscis is bare but the remainder bears eight spirally arranged rows of hooks, there being 14-17 hooks in each row. Each hook is recurved, from 31-36  $\mu$  in length, and has but a single root. Proboscis receptacle is a double walled muscular sac with retractors passing through the posterior extremity. The outer wall of the receptacle is disposed in spiral bands.

The limnisci are narrow, contorted and about one-seventh as long as the body. The testes are situated posteriorly where they fill almost the entire body cavity, one being posterior to the other and close to it. Each testis is an elongated slightly curved body somewhat flattened dorsoventrally and measures from 1.3 to 1.6 mm. in length by 0.58 to 0.61 mm. in breadth. The prostatic glands are situated posterior to the testes very near the end of the body. They are eight in number and form an elongate group 0.8 to 0.84 mm. long and 0.35 to 0.38 mm. in greatest breadth.

Eggs are present in large numbers in small females 45 mm. long as well as the largest specimens. They are spindle-shaped with sharply rounded ends and measure 59 to 65  $\mu$  in length and 20 to 22  $\mu$  in width and have thin wrinkled shells when deposited. In younger females the shells are thicker and smooth giving the eggs a width of 26 to 28  $\mu$ . Eggs are only slightly segmented when deposited.

The above-described *Acanthocephala* possesses the characters of the genus *Moniliformis*, sub-order Echinorhynchidea. It resembles the species *Moniliformis moniliformis* (25) in general body shape and the arrangement of the various organs, but there are sufficient differences in the relative body measurements, the number and arrangement of the proboscid hooks and the shape

of the testes to warrant the creation of a new species, in the opinion of the writer, and the name *spiradentatis* is suggested.

*Type host.* *C. tridecemlineatus* (in stomach and intestine).

*Type locality.* Manitoba, Canada.

*Weinlandia citelli*, n. sp.

(Figs. 15, 16).

*Type species.* *Weinlandia macrostrobilodes* Mayhew, 1925. Length 15 cm. and greatest width 2.8 cm. The anterior portion is very much attenuated, the width just posterior to the scolex being 175  $\mu$ , while 27 cm. behind, where the sexual organs are first fully formed, it is 277  $\mu$ . Increase in width is gradual and the first gravid proglottid measures 1.3 mm. with a continuous increase toward the posterior end until a maximum width of 2.8 mm. is reached. There is scarcely any neck since the slight constrictions indicating the beginning of strobilization are evident about 180  $\mu$  behind the scolex. The length of an anterior proglottid is about one-eighth of the width, but when the sex organs begin to develop there is an increase in length with regard to width so that at a point where they are 0.94 mm. wide the length of each proglottid is 157  $\mu$ . In the midbody region gravid segments of 1.57 mm. in width measure 330  $\mu$  in length. The genital pores are unilateral and on the right side.

The scolex is but little wider than the anterior end of the strobila and with the suckers measures 245  $\mu$  in width by 157  $\mu$  dorsoventrally. The suckers are elliptical and measure 113  $\mu$  in length and 87  $\mu$  in breadth with a prominent rim about 21  $\mu$  wide. The rostellum is a rather indefinite triangular structure at the anterior termination, about 38  $\mu$  long when extended.

Three testes are present, two lie at the posterior margin of the proglottid about equidistant from the ovary, one being poral and the other antiporal. The third is anterior and lateral to the posterior antiporal testis and may lie somewhat dorsal or ventral to it in some segments. The testes are oval or spherical in shape and in segments 930  $\mu$  in width measure 143  $\mu$  in length and 113  $\mu$  in width. The vas efferentia are very small tubes not readily discernible in *in toto* mounts but they appear to arise from the anterior margins of the testes. Those from the two antiporal testes unite to form a common duct which is joined by the one from the poral side. The cirrus sac is one of the most conspicuous structures of the proglottid and is a fusiform, slightly curved structure about 157  $\mu$  in length opening a little posterior to the middle of the proglottid on the right side.

The cirrus is a thin rod-like structure arising at the inner end of the cirrus sac and terminating in a clavate expansion which curves sharply anteriorly after passing through the opening.

The vagina is an indistinct, somewhat twisted tube which runs anteriorly from the shell gland for a short distance and then turns laterally passing with the vas deferens below the excretory vessels.



The ovary is deeply divided into eight oval or spherical lobes forming a crescent-shaped mass in the middle of the non-gravid proglottid. The vitelline gland is compact, almost spherical and lies in the concavity on the posterior side of the ovary, while the shell gland is also rounded and situated ventrally beneath the vitelline gland.

The uterus in the non-gravid proglottids is a small irregular sac situated anteriorly and to the left of the ovary. In gravid proglottids it extends almost to the edges of the segment, its margin being thrown into folds so deep in some places that a number of lobes are formed. There are two excretory vessels on each side of the strobila. They are narrow and almost straight tubes, the dorsal being slightly larger and median to the ventral. On the right side they pass close to the inner end of the cirrus sac.

Eggs oval to almost spherical in shape and measure  $78-86\ \mu$  in length by  $59-65\ \mu$  in width. There is a thin outer shell and thick albuminous layer about one-quarter of the diameter of the egg in thickness, surrounding the inner shell and hexacanth embryo.

The presence of three relatively large testes in each proglottid, sac-like uterus, unarmed rostellum and unilateral genital pores places the cestode described above in the family Hymenolepididae (Ariola, 1899) (18). The arrangement of the three testes, two being posterior and the third being anterior and lateral to the posterior antiporal testis supports the opinion that it is a member of the genus *Weinlandia* (Mayhew, 1925) (18). Twenty-seven species of this genus have been recorded from birds by Mayhew (18), but none from rodents of the genus *Citellus* as far as the writer is aware. There appears to be a definite host specificity among members of this family and it is considered unlikely that an avian species would be found in a mammal. The only Hymenolepid recorded from *Citellus* sp. to the author's knowledge is *H. megaloon* (Linstow, 1901) (16), which differs from the above species in the possession of a conical scolex with shallow bothria and three testes arranged in a posterior row. On these grounds it is considered as a hitherto unrecorded species and the specific name *citelli* is suggested.

*Type host.* *C. tridecemlineatus*, *C. richardsoni*, *C. franklini* (in stomach and intestine).

*Type locality.* Manitoba, Canada.

*Prochoanotaenia spermophili*, n. sp.

(Figs. 17, 18).

Small worms of not more than 10 cm. in length. The anterior end is somewhat attenuated but the increase in body width is gradual. Behind the scolex the body width is  $334\ \mu$ , while in the midbody region sexually mature but non-gravid segments measure about  $720\ \mu$  in greatest width. Gravid segments at the posterior end measure about 1 mm. in width. Distinct strobilization begins almost directly behind the scolex leaving a neck of only about  $740\ \mu$  in length. The anterior proglottids are narrower at their

anterior ends, being about three-quarters of the width of the posterior ends, while the length is about two-thirds of the greatest width. In sexually mature but non-gravid segments the posterior width is about  $710\ \mu$ , the anterior about  $595\ \mu$  and the length about  $635\ \mu$ . Gravid segments are about the same width at each end but are slightly expanded in the mid-region. Those with a width of 1.08 mm. have a length of about 1.45 mm. The genital pores are irregularly alternate usually in two's or three's.

The scolex is somewhat narrower than the neck and with the suckers measures about  $332\ \mu$  in width and  $227\ \mu$  in thickness. The suckers are on the dorsal and ventral surfaces and are prominent circular cups  $122\ \mu$  in diameter with depressions  $66\ \mu$  in diameter. The rostellum in a distended condition measured  $105\ \mu$  in length and is a fungiform structure bearing on its expanded end a single circle of very fine hooks each with a single root. The rostellum is retractible into a dagger-shaped muscular bulb  $315\ \mu$  in length and  $105\ \mu$  in greatest width.

Testes are about  $70\ \mu$  in diameter in proglottids  $780\ \mu$  wide and are oval or spherical in shape. There are 20–25 in each proglottid and all are post-ovarial in position. The vas deferens is first discernible just anterior to the ovary where it runs anteriorly for a short distance and then follows a convoluted course laterally passing ventral to the excretory vessels to the cirrus sac. The cirrus sac is a fairly conspicuous clavate-shaped structure extending obliquely inward about  $140\ \mu$ . In sexually mature segments it opens on a prominent lateral elevation about one-quarter of the length of the segment from the anterior end. The cirrus is convoluted and the blunt outer end projects through the opening in gravid segments.

The vagina is an indistinct arched tube running in an antero-lateral direction. Its outer end is almost parallel to the cirrus sac and opens immediately posterior to it. Like the vas deferens it passes ventral to the longitudinal excretory canals.

The ovary is divided into 10 to 14 finger-like lobes radially arranged in a semicircle. It lies in the anterior half of the segment and is somewhat antiporal in position. The vitelline gland is a compact uni- or bilobed structure almost median in position and with an anterior angle or concavity close to which lies the spherical shell gland.

The uterus is an irregular sac anterior to the shell gland and dorsal to the ovary in sexually mature but non-gravid segments, but is replaced by egg capsules in the gravid ones. The excretory vessels run parallel to, and are about one-fifth of the width of the segment from the lateral margins.

*Eggs.* Very numerous in gravid segments and are  $56$ – $62\ \mu$  long by  $42$ – $46\ \mu$  wide. There is a thin smooth outer shell and a thick albuminous layer surrounding the inner shell and embryo which is  $31$ – $33\ \mu$  in diameter.

The possession of marginal genital pores, post-ovarial position of the vitelline gland, non T-shaped rostellar hooks and the post-ovarial arrangement of the testes identifies the above cestode as a member of the family Dile-

pididae. The presence of an unstable uterus which is replaced by egg capsules places it in the subfamily Dipylidinae. That it belongs to the genus *Prochoanotaenia* (19), is supported by the possession of a single set of genitalia, absence of spines on the neck, position of the genital ducts below the longitudinal excretory vessels, alternate genital pores and a rostellum with a single row of hooks. A search of the literature reveals the most closely related species from small mammalia to be *Taenia blanchardi* (20), recorded from *Talpa europaea*. This differs from the species described here in the armature of the rostellum, and the relative size of the suckers. *Taenia blanchardi* also differs in that the ovary is arranged around the vitellaria while in *Prochoanotaenia spermophili*, n. sp. the vitellaria lie posterior to the ovary. To the writer's knowledge no member of this genus has been recorded from *Citellus* and the name *spermophili* is suggested.

*Type host.* *C. tridecemlineatus*, *C. richardsoni*.

*Type locality.* Manitoba, Canada.

### Discussion

*Dermacentor venustus* was found to be quite common in the adult stage on the majority of the *Citellus* species examined in the early summer. This tick plays an indispensable part in the transmission of *Pasteurella tularensis* and *Dermacentroxenos rickettsi* the causative organisms of tularemia and Rocky Mountain spotted fever (17, 22). These diseases have always been confined to regions where this tick is abundant since its host relations, feeding as it does during the early stages of its development on rodents which serve as reservoirs for the diseases and as adults on man and higher animals, makes it pre-eminently suited for the role it plays.

With a large gopher population as a potential reservoir and the widespread occurrence of such a potential transmitter as *Dermacentor venustus*, the outbreak of an epidemic would appear to depend only on the introduction of the virus in an infected animal or tick. *Dermacentor venustus* has also been responsible for a number of cases of tick paralysis (4).

*Ceratophyllus bruneri* was found to infest almost 100% of the gophers examined. No direct pathological condition results from the bite of this insect but bubonic and pneumonic plague of man, common in numerous parts of the world, owe their persistence to the presence of animal reservoirs in the form of small rodents and their transmission from one host to another to the fleas of these rodents (15). Climatic conditions in western Canada are sufficiently similar to those of the endemic plague areas to spread this disease if once introduced. *Ceratophyllus bruneri* may also act as the transmitter of *Trypanosoma citelli* and as the secondary host of the tapeworm *Weinlandia citelli*.

*Warrenius bifurcatus* ranks first as a pathogenic organism of all the parasites listed in this paper. It occurs in numbers up to 80 in a single host and is always found firmly attached with the anterior end deeply buried in the

sub-mucosa or circular muscle layer of the gut (Plate I, Fig. 1). They are blood feeders and tissue mutilators and their presence is always accompanied by a hemorrhagic and necrotic condition of the parts attacked. Chronic inflammation and sometimes perforation of the intestine results from the presence of these worms. It infests about 54% of the entire gopher population with a mean number of 24 worms per host, thus constituting a serious menace to the health of these rodents.

*Spirura infundibuliformis* is another tissue-mutilating form. It is found in large numbers attached through a loop of tissue at the cardiac end of the stomach, the struma preventing it from becoming dislodged. Large worms such as *Weinlandia citelli* and *Moniliformis spiradentatis* seriously interfere with the normal functioning of the intestine and may almost completely block it in some cases (Plate I, Fig. 2). In such a case the intestinal wall becomes stretched and thin with a general emaciated appearance.

### Parasitism and Host Abundance

The effect of the environment on an animal species, such as weather conditions, food supply and associated organisms, may be manifest either directly in the longevity of the individual or indirectly in its fecundity. Members of the genus *Citellus*, due to their hibernating habit, are well adapted to withstand winter and other unfavorable weather conditions in western Canada unless interfered with from some other source. Since they live in what are now agricultural areas, food is very abundant during their active season and they are practically free from the attacks of such predators as the coyote, fox, badger, hawk and owl. It must rest then with pathogenic organisms to bring about the condition which so regularly depletes their numbers.

Parasitic infestation, as shown by Boughton (3), depends on the rate of egg production, life-cycle stages, presence of a secondary host where necessary and the chance of gaining entrance to the host. Overcrowding, resulting from rapid increase due to favorable weather and food conditions and the absence of predators, more than proportionately increases the possibility of the parasites gaining entrance to the host. Parasitic infestation also depends on the specific resistance of the host in a manner similar to bacterial infection (5). The detrimental influence of the Helminths listed in this paper is increased by the fact that they are metabiotic and several species can infest the host at the same time; in fact the greatest number of a single species was often found in the presence of other parasites. The presence of one species appears to lower the host's resistance and facilitate the existence of others. For example a single specimen of *C. tridecemlineatus* showed infestation as follows:—*Weinlandia citelli*, 8; *Moniliformis spiradentatis*, 19; *Spirura infundibuliformis*, 48; A single specimen of *C. richardsoni* also showed;—*Warrenius bifurcatus*, 62; *Weinlandia citelli*, 20; and *Prochoanotaenia spermophili*, 14.

### Acknowledgments

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FIG. 1. Transverse section of intestine with worms attached showing destruction of villi and inflamed sub-mucosa. FIG. 2. Transverse section of intestine with *Weinlandia citelli* in situ.



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## STUDIES ON BACILLUS CALMETTE-GUÉRIN (B.C.G.) AND VACCINATION AGAINST TUBERCULOSIS<sup>1</sup>

BY E. A. WATSON<sup>2</sup>

### Abstract

Vaccination trials and biological studies of B.C.G. have been carried on for a period of over eight years and are still continuing. Under conditions of continuous exposure to natural infection through cohabitation with tuberculous animals, trials varying in duration from two months to four and one-half years have been completed on 44 vaccinated cattle and 28 unvaccinated controls. In trials of short duration the percentage of cattle free from tuberculosis is slightly in favor of the vaccinated. In all cattle over two years of age tuberculosis was present to a greater or lesser degree. Judged by slight, moderate and extensive tuberculous involvement, there is some evidence of a greater resistance in the vaccinated cattle up to two and a half years of age, 26% of which showed extensive generalized tuberculosis, as compared with 53% of the unvaccinated. But in the age group ranging from two and a half to four and a half years no greater resistance is found in the vaccinated than in the unvaccinated cattle. It has not been possible to demonstrate a true lasting immunity by this method of vaccination, and such increased relative resistance as B.C.G. may confer during the early months of life declines and soon disappears, and fails to protect cattle exposed for two years to natural sources of infection from developing typical tuberculosis.

The attenuated virulence and potential pathogenicity of B.C.G. have been studied for a period of over eight years in three original strains received in the years 1924, 1925 and 1927, and in the cultural descendants of each strain up to the year 1932. Each strain proved to possess an unfixed, potential virulence capable of exaltation on the one hand, and of complete attenuation or reduction on the other. This virulence, manifested but rarely and only in the earlier descendants, 1924-1928, declined under serial cultivation and periodic return to the special bile-potato media and apparently died out in the 1928-1929 generations, for none of the subsequent descendants tested proved capable of causing progressive reinoculable tuberculosis in laboratory animals.

A number of tuberculosis research studies engaged in by the Dominion Department of Agriculture were incorporated into the research program of this Associate Committee on Tuberculosis as drafted and adopted in the year 1925. These include studies relating to vaccination with living tubercle bacilli, and especially investigations of the claims made in respect to B.C.G. vaccination of cattle, begun more than eight years ago, and continuing under the direction of the writer and in collaboration with Drs. McIntosh and Konst up to the present time. Several interim reports have been made to this committee at previous meetings, and the present report is, more or less, a summary of (1) experimental trials of vaccination and resistance of cattle under exposure to natural sources of tuberculosis infection, and (2) experiments relating to the possible pathogenicity and virulence of B.C.G.

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<sup>2</sup> Chief Pathologist, Animal Diseases Research Institute.

### **Repetition of the Vaccination Experiments made by Calmette and Guérin, and the Determination of the Duration of the Immunity\***

In reporting, interpreting, judging and evaluating the results of B.C.G. vaccination of cattle, careful consideration must be given to the conditions under which the vaccination and immunity trials are conducted and to which the animals are subject from the time of birth, and for the whole duration of the experiment. Apart from the variable degree of natural susceptibility and resistance in individual animals, many factors may enter, such as methods of handling calves at birth, of feeding and housing, and the environment in which the animals are placed; the sources of infection to which they are exposed, month by month, year by year; the severity, frequency and duration of those exposures; breeding, pregnancy and lactation, etc. Such conditions can be controlled only in part; and in practice will vary greatly from time to time and even from month to month, in degree and in kind, and have variable effect upon resistance and experimental results. A great deal of the conflicting results reported by different authors in different countries, or by the same author with different groups of animals spread over a period of years, is, in all probability, due in large measure to the different and varying conditions under which the trials are conducted, and to different prevailing factors. It appears, therefore, very necessary to state as clearly as possible the known conditions of the experiments and at the same time bear in mind that variable factors remain unknown. The cattle vaccination studies under the auspices of this committee have been made under conditions as here set forth:

1. The original cultures of B.C.G. were obtained direct from France, from Professors Calmette and Guérin. Subcultures were propagated successively on potato media and Sauton's special media, interrupted at each 10th to 12th generation by a return to ox-bile-potato media as recommended.

2. The vaccines injected into cattle were prepared from subcultures made under the conditions stated in paragraph No. 1. With each lot of prepared vaccine inoculated into cattle, pathogenicity tests were made on guinea pigs.

3. Each year, from 1925 to 1931, one or more groups of new-born calves were inoculated within the first few days of life with the prescribed dose of B.C.G., subcutaneously, in the dewlap.

4. Each year, from 1925 to 1931, one or more groups of calves have been left as controls, *i.e.*, unvaccinated, but otherwise subject to the same conditions as the vaccinated.

5. Calves born in the years 1925 and 1926 were vaccinated and exposed to infection without special safeguards or change in ordinary farm methods, and in accordance with the conditions prescribed at that time.

*\*(Research Program, Associate Committee on Tuberculosis, Item 5 (d).)*

6. Calves born in the years 1927 to 1931 were strictly isolated and fed only pasteurized milk from birth, for a period of one to two months after vaccination with B.C.G., in accordance with the revised conditions laid down in 1927.

7. Vaccinated calves not disposed of during the first year of life were re-vaccinated, at intervals of approximately one year, until death or slaughter.

8. Exposure to infection was by natural means, first through the ingestion of raw milk from cows which included one or more tuberculous animals, and then by cohabitation with tuberculous cattle in stables and in pastures. In order to equalize hazards of exposure, the stable locations of the vaccinated, unvaccinated and tuberculous cattle were changed from time to time, and during the summer months the animals mingled together freely in small pastures or outside enclosures, in which they were fed from large open racks or mangers.

9. The duration of the exposure and immunity trials have varied from a few months up to four and a half years.

10. As a rule, the young males were first disposed of, within the first or second year of life, and later on the heifers after one or more periods of gestation and lactation.

11. Laboratory post-mortem examinations have been conducted in each case, and the search has been much more thorough than is customary or is possible under abattoir conditions.

12. Microscopical examinations and test inoculations have been made of the vaccinal nodes or lesions present in the dewlap, the lesions found in other tissues and organs, and of lymphatic gland tissues in which no visible lesions could be found.

The summaries and observations that follow concern only cattle in which the resistance or immunity has been tested by exposure to natural sources of infection under the conditions already stated.

In the above category there are 107 cattle, of which 35 are living and held for further trial and observation, and 72 are dead. The latter, consisting of 44 vaccinated cattle and 28 unvaccinated controls, died or were killed at an age ranging from two months to four and a half years and, in each case, were subject to necropsy under laboratory conditions, microscopical examinations and guinea pig test inoculations. Regardless of age or the duration of the trial, it was found that nine, or 20.5%, of the vaccinated, and six, or 21.4%, of the unvaccinated cattle were free from tuberculosis, and that tuberculosis was present in the remainder of each group.

The number and approximate percentage of cattle, according to (a) age grouping, and (b) cumulative results, are shown in Table I.

A slight difference in favor of the vaccinated cattle up to 18 months of age may be noted, but with accumulating numbers and results, and when

all cattle up to the age of four and a half years are included, the percentage is practically the same for the vaccinated and for the controls.

Actually, the cattle free from tuberculosis in both vaccinated and unvaccinated groups were under two years of age, and all cattle over two years of age showed tuberculosis to a greater or lesser degree.

TABLE I  
VACCINATED AND UNVACCINATED CATTLE FREE FROM TUBERCULOSIS

Age groups	Number of cattle			Free from tuberculosis, %	
	B.C.G.	Controls	Total	B.C.G.	Controls
Up to 1 year	5	4	9	100	75
1 to 1½ years	8	3	11	25	0
1½ to 2 years	14	9	23	14	33
2 to 2½ years	9	4	13	0	0
2½ to 4½ years	8	8	16	0	0
All cattle	44	28	72	20	21
(Cumulative)					
Up to 1 year	5	4	9	100	75
Up to 1½ years	13	7	20	54	43
Up to 2 years	27	16	43	33	37
Up to 2½ years	36	20	56	25	30
Up to 4½ years	44	28	72	20	21

### Comparative Resistance to Tuberculosis of Vaccinated and Unvaccinated Cattle

The comparative and relative resistance as indicated by the activities of tuberculous lesions and the extent of their involvement have been studied. Extreme variability in the type or stage, extent and location of the tuberculous processes is as noticeable in the vaccinated as in the unvaccinated groups, in each of which some animals show only small foci of infection limited to one or several groups of lymphatic glands; others have revealed more scattered lesions of a progressive type; and others, the classical picture of advanced generalized tuberculosis.

For convenience and to permit of a simple comparison, the degree and extent of tuberculosis is indicated in Table II by three subdivisions: slight, moderate, and extensive.

There is little difference in the incidence of tuberculosis in the vaccinated and unvaccinated groups, but there is some evidence that in the vaccinated cattle there is a greater degree of resistance up to two and a half years of age, and as indicated by 26% of the vaccinated and 53% of the unvaccinated with extensive involvement. It is noteworthy, however, that in the age group, two and a half to four and a half years, the percentage of cattle with slight, moderate and extensive tuberculosis, namely, 25, 25 and 50%, is identical for the vaccinated and unvaccinated cattle.

TABLE II

VACCINATED AND UNVACCINATED CATTLE WITH TUBERCULOUS INVOLVEMENT

Age group	Number of cattle	With tuberculosis, %			
		Slight	Moderate	Extensive	Total
Up to 1½ years	B.C.G. 13	38	8	0	46
	Controls 7	28	28	0	56
1½ to 2½ years	B.C.G. 23	30	35	26	91
	Controls 13	8	15	53	76
2½ to 4½ years	B.C.G. 8	25	25	50	100
	Controls 8	25	25	50	100
All cattle up to 4½ years	Totals:—				
	B.C.G. 44	32	25	22	79
	Controls 28	18	21	39	78

In brief, it may be said: (1) that in these groups of cattle, the greater degree of resistance of the B.C.G. vaccinated animals is best indicated in animals up to two to two and a half years of age; and (2) that the resistance in vaccinated animals which have reached breeding age, two and a half years and up, and after one or more periods of gestation and lactation, is more or less parallel or comparable with the resistance of unvaccinated cattle.

In other words, increased resistance following B.C.G. vaccination of newborn calves diminishes as the animals continue in cohabitation with tuberculous cattle and approach maturity, and is apparently lost in young cows which are being bred and milked.

There is evidence that B.C.G. vaccination causes or increases a relative resistance which may delay the development of infection and disease, particularly during the early period of life, but in most cases this is transient and declines as the animals approach maturity, and it has not been sustained by annual revaccination.

The results have varied considerably in individuals and in groups of animals which, of course, is to be expected in such a chronic disease as tuberculosis where the conditions of life and exposure to infection and reinfection on the one hand, and natural susceptibility and resistance on the other, are such variable and important factors.

One or two cases of more than usual interest may be mentioned. For example, a B.C.G. vaccinated animal, No. 188, was killed at the age of two years and ten months when in a moribund condition. During the last three months of life this animal showed increasing difficulty in breathing and swallowing, developed a very large swelling in the submaxillary region, and became much emaciated. At necropsy, the retropharyngeal glands were uncovered as an enormous mass of caseo-purulent tuberculous lesions pressing upon the pharynx and larynx. There were no other visible lesions in the body except a small focus in the mesenteric gland.

Another B.C.G. vaccinated animal, No. 218, manifested clinical advanced tuberculosis and was moribund at two years and nine months of age. In addition to typical lesions of advanced pulmonary and lymphatic gland tuberculosis, the larynx and trachea showed extensive tuberculous ulcerations and caseo-purulent nodules. (See Plates I and II.)

The evidence thus far accumulated in the trials and experiments conducted over a period of seven to eight years, shows that approximately 80% of the cattle killed at ages ranging from calthood to maturity developed tuberculosis to a variable extent while living under conditions of exposure to natural infection and reinfection, with approximately the same incidence in the vaccinated and in the unvaccinated, but at a slower rate of development and progress, especially in early life, in the vaccinated animals, thus indicating some increased resistance. This, possibly, would be more in evidence if and when B.C.G. vaccination is applied under conditions where exposure to infection is slight and infrequent and resistance is not weakened by the stress and strain to which breeding cattle, especially dairy cattle of high production, are subjected.

### **The Attenuated Virulence and Potential Pathogenicity of B.C.G.**

Our experiments and studies relating to the attenuated virulence and the properties of B.C.G. have been made on the descendants of three original strains of B.C.G. received direct from France.

The first strain, herein referred to as B.C.G. 80, was forwarded by Dr. Guérin from the Pasteur Institute, Lille, and was received in November 1924.

The second strain, B.C.G. 17, came from Professor Calmette's laboratory, Pasteur Institute, Paris, September 1925.

The third strain, B.C.G. 346, also came from Calmette's laboratory, December 1927.

It is important, for several reasons, as will be seen later on, to keep in mind the fact that the work has been done with three parent strains and their descendants, the dates or years of their origin, 1924, 1925, 1927, and their succession and maintenance as separate B.C.G. strains up to the year 1932.

Tests of the descendants of these culture strains of B.C.G. have been spread over a period of eight years and have furnished an enormous number of data which are now being compiled in tabulated form and subjected to careful analysis. This analysis is bringing out features of special interest, some of which are presented in the following outline or summary.

1. Tests of 145 cultures of B.C.G., representative of subcultures descending to the 121st generation, have been made on over 500 guinea pigs.

In approximately 56% of the guinea pigs there was no evidence of tuberculosis at necropsy.

Slight or localized lesions, or apparently arrested or healed lesions, were present in 40% of the guinea pigs.

Typical tuberculosis, reinoculable from animal to animal, in series, or by reisolation in culture and subsequent inoculation, developed in 4% of the guinea pigs.

(Further reference to slight or arrested lesions may be omitted as such may, and probably do for the most part, fall into that category stressed by Calmette as undergoing healing, absorption and retrogression.

2. Only 6, or 4%, of the 145 B.C.G. cultures tested proved to be virulent for guinea pigs.

(It so happens, as a coincidence only, that the percentage of virulent cultures and the percentage of guinea pigs developing tuberculosis in all inoculation tests are the same, namely, 4%).

But 21, or 42%, of the 50 guinea pigs which received inoculations of these six virulent descendants, developed typical tuberculosis.

3. Each of these six virulent descendants proved to be capable of causing a progressive tuberculosis, reinoculable from animal to animal.

Four of these substrains of proved virulence were reisolated in culture, in the course of serial animal passage, and repeatedly proved pathogenic for guinea pigs and rabbits, and of a type and virulence corresponding to the usual bovine type.

4. The period in which these six virulent cultures were forthcoming dates from the end of the year 1924 to 1928, and for each parent or original strain is subdivided as follows:

(a) B.C.G. 80,—3 virulent descendants—1924 to 1926.

(b) B.C.G. 17,—2 virulent descendants—1925 to 1927.

(c) B.C.G. 346,—1 virulent descendant—1928.

None of the later descendants tested,—of B.C.G. 80 since 1926, of B.C.G. 17 since 1927, and of B.C.G. 346 since 1928,—proved to be virulent for guinea pigs or capable of causing a progressive, reinoculable tuberculosis.

5. Approximately 17% of test inoculations for virulence were positive in the year 1925; 10% in the years 1926 and 1927; less than 5% in the year 1928; and zero, or 0%, 1929-1932.

6. *B.C.G. strain 80*, the first strain received from France, 1924, has been tested at intervals between the 1st and 121st generation. Twenty-nine cultures, each of a different generation, were tested, and three, or 10.3%, proved virulent for 9.8% of the test guinea pigs. These three virulent cultures were produced between the 1st and 18th generation and no virulent cultures have since been produced from the 19th to the 121st generation.

*B.C.G. strain 17*, the second strain received from France, 1925, has been tested at intervals between the 14th and 107th generation. Seventy cultures were tested and two, or 2.8%, proved virulent for 2.4% of the test guinea pigs. These two virulent cultures represent the 22nd and 36th generation respectively, and no virulent culture has been produced in the succeeding 71 generations, *i.e.*, from the 37th to 107th generation.

*B.C.G. strain 346*, the third strain received from France, 1927, has been tested at intervals between the 13th and 77th generation. Forty-six cultures or descendants of different generations were tested and only one, or 2.1%, proved virulent for 0.64% of the test guinea pigs. This virulent culture represents the 13th generation, and all subsequent cultures up to the 77th generation have been negative.

7. The data and figures covering the entire eight-year period of culture and virulence testing show that the number and percentage of virulent sub-cultures derived from each parent strain, and also the number and percentage of positive inoculation tests, declined from the first year and had fallen to zero at the end of the fourth year (1924-1928) where it has remained for a further period of four years (1928-1932).

8. From the commencement, B.C.G. has been regularly and continuously reproduced in serial transfer upon the culture media, and in accordance with the procedure recommended by Calmette, being regularly returned to bile-potato media at stated intervals.

9. The virulence as determined in the small percentage of these early descendants (1924-1927) required a long period for its manifestation in the original test animal and, in some cases, did not reach full or average virulence until the second or third serial animal passage.

10. Experiments made to bring back or to restore the virulence lost by the B.C.G. descendants since 1928, by means of "dissociation" into different colony types, by "deep growth" methods of culture, by adding serum to the media, and in other ways, have not met with any certain success.

### Conclusion

A careful analysis of the history of these strains of B.C.G. and of the virulence and pathogenicity tests of their descendants continuing over a period of eight years leads, in the opinion of the writer, to but one clear and certain conclusion:—that the Calmette-Guérin bacillus or "B.C.G.", as issued in the years 1924-1927, was still in process of attenuation, that its properties were not then completely and hereditarily fixed, and that it still retained a low, uncertain and potential virulence, which, in the one direction, by animal passages and reisolation has been exalted to and maintained at a high degree of virulence; and, in the other direction, in the course of several years of continuous culture and reproduction on the special laboratory media designed by the originators of B.C.G. for its attenuation, has declined and vanished.

It is surely significant that with cultures of B.C.G. that were being distributed for study in the years 1924-1927, not only the writer and his coworkers but a number of investigators in different countries reported experiments and evidence in disproof of the claim that B.C.G. was completely and absolutely devoid of virulence and that its properties were hereditarily fixed. Moreover, in 1927, Calmette recommended, as a safety precaution, that B.C.G. be returned to bile-potato at stated intervals.



It may here be recalled (Calmette and Guérin, Ann. inst. Pasteur, May, 1924) that B.C.G. originated from a highly virulent bovine strain of tubercle bacilli and that its virulence was slowly attenuated first for one species of susceptible animal and then for another, successively, during 13 years of cultivation on bile-potato, on which it apparently lost its property of tuberculin production, and since that date (January 5, 1921: 230th passage) was kept on ordinary glycerinated potato, "with the object of definitely fixing its properties"; and that under the latter conditions it regained its tuberculogenic properties.

It need occasion no surprise therefore, to discover that B.C.G. still retained some potential virulence, even though rarely manifested, and that this has been suppressed only by a continuation of the special cultural methods for its attenuation over a further period of years.

At this present time we can make the statement on positive proof evidence that the parent strains of B.C.G., as they came to us, and their early descendants possessed an unfixed potential virulence capable of exaltation on the one hand and of further and complete reduction on the other, each of which has been accomplished; and, on negative evidence, that the later descendants of the same strains (1929-1932), by continuous reproduction and periodic return to bile media in accordance with Calmette's recommended procedure, have become incapable of causing progressive, re inoculable tuberculosis in laboratory test animals.

### EXPLANATION OF PLATES

Types of tuberculosis in cattle vaccinated with B.C.G. as newborn calves and raised in a tuberculous environment.

#### PLATE I

Fig. 1. Laryngeal and tracheal tuberculosis (No. 218).

#### PLATE II

Figs. 1 and 2. Pulmonary and hepatic tuberculosis (No. 218).

Figs. 3 and 4. Glandular tuberculosis (No. 188).

*Animal No. 218.* Born May 19, 1929. Isolated 40 days. B.C.G. vaccinations: May 27, 1929; June 28, 1930; June 29, 1931. Slaughtered March 1, 1932, at two years and 9 months of age. Large caseous retropharyngeal lymph nodes; caseo-purulent nodes and cavities in the lungs and liver, tuberculous ulceration of larynx and trachea.

*Animal No. 188.* Born July 4, 1928. Isolated 40 days. B.C.G. vaccinations: July 9, 1928; August 8, 1929; September 4, 1930. Slaughtered May 7, 1931, at two years and 10 months of age. Extensive tuberculosis of retropharyngeal lymph nodes.







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# THE REVERIFICATION OF A FOUR-METRE RULE<sup>1</sup>

By R. H. FIELD<sup>2</sup>

## Abstract

An investigation is described in which a determination was made of the length equation of a four-metre invar rule used as a basis for measuring tapes. A short description of the rule and its associated apparatus is given. The investigation demonstrated that different rules made at the same time from the same ingot of invar do not necessarily have identical thermal coefficients. It was also found that the temperatures of two rules of different cross section differed considerably even in a room where the air temperature was changing only at a very slow rate *i.e.*, 1° C. in two or three hours.

## Introduction

The base\* employed for standardizing measuring tapes in the Division of Physics and Engineering of the National Research Laboratories, Ottawa, consists of a series of concrete piers rising from a monolith beneath the floor and spaced at four-metre intervals. Each pier carries a bracket on which is mounted a horizontal graduated plate or bench mark. Electric radiators under thermostatic control, together with thick insulated walls and the absence of windows, permit a very steady temperature to be maintained throughout the room.

## The Four-metre Rule

To measure the intervals between adjacent bench marks an invar rule, four metres in length, is supported on a special carriage which can be moved along a track in front of the piers. This track also serves to support microscope stands and other subsidiary apparatus used in comparing tapes with the base. The four-metre rule and carriage are illustrated in Fig. 1. At the right-hand side can be seen one of the brackets carrying the bench marks.

Naturally it is important that the length of the four-metre rule be accurately known at all temperatures at which tapes are measured, *i.e.*, within the range 0° to 30°C.

Invar, while accepted as the best material for constructing rules of the type under discussion, suffers from the drawback of being subject to secular dimension changes, particularly in the early part of its life. There is doubt also as to whether the thermal equations of nickel steels remain constant during the time that these secular changes occur.

It was originally proposed to send the rule periodically to the International Bureau of Weights and Measures, Sèvres, for verification, but its size is such that there would be an element of risk in this course owing to the adverse

<sup>1</sup> Manuscript received July 21, 1933.

*Contribution from the Physical Testing Laboratories, Division of Physics, National Research Laboratories, Ottawa, Canada.*

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\*For a complete description of the tape-verifying base, see Ref. (1).

effect of shocks that might be received during the journey. Consequently it was decided to acquire one or more one-metre rules and construct apparatus whereby these could be used to measure the four-metre rule as required, metre by metre.

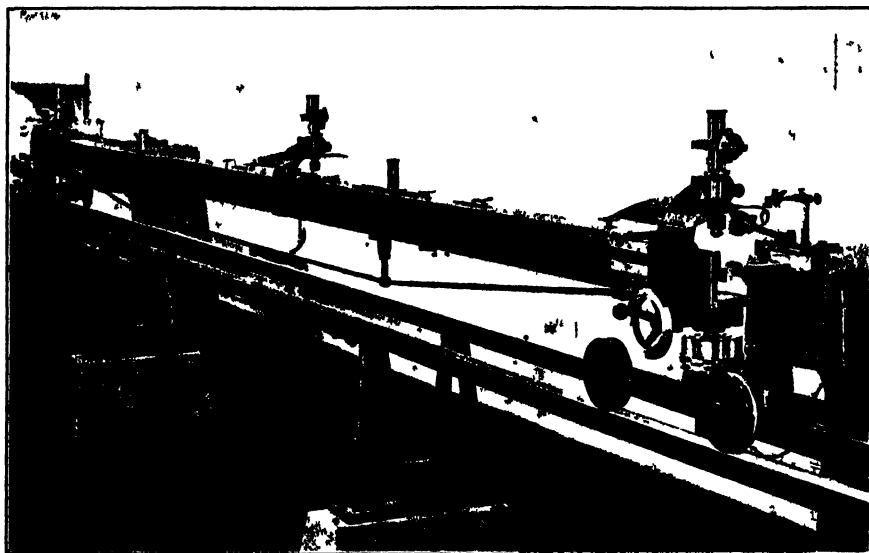


FIG 1 Carriage for the four-metre rule employed to measure the tape-standardizing base at the National Research Laboratories, Ottawa. The right end of the rule is just visible near the bench mark bracket.

The section of the four-metre rule is shown in Fig 2. The graduations defining its length are ruled to the edge of the exposed neutral surface in order that the rule can be placed opposite the bench marks in such a way that the graduations on rule and bench mark are seen simultaneously in the fields of the observing microscopes. This permits a measurement to be made of the interval between the principal graduations in question.

Unfortunately the manufacturers found difficulty in machining the rule perfectly straight, and it is appreciably curved in both the horizontal and the vertical planes. The ordinate between the chord joining the edge at the zero and the four-metre graduations and the point of maximum deflection is about 2 mm. An appreciable correction has to be applied, therefore, to reduce the sum of the lengths of the four one-metre chords, as determined by comparison with a one-metre rule, to the length in the direction of the four-metre chord.

The magnitude of this correction has been determined by a separate investigation (1, p. 25). Its value is  $3.6 \mu$ , and there is no evidence that it varies with temperature by an appreciable amount.

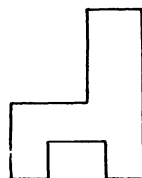


FIG 2 Cross section of the four-metre rule (one-half full size).

### Thermal Equation of the Four-metre Rule

The rule was originally verified by the International Bureau of Weights and Measures in 1913. To determine the thermal dilatation, observations were made by the Bureau on a sample rule, one metre long, made from the same ingot as the four-metre rule. It was assumed that the four-metre rule had the same coefficients as the sample. The thermal equation given in the certificate issued by the Bureau is—

$$\alpha = (1\ 738\theta - 0.00509\theta^2) 10^{-6} \quad (1)$$

where  $\theta$  is the temperature on the hydrogen scale.

When the rule was later received in Ottawa an extended series of verifications was made of a number of laboratory standard tapes. It was found in the hot summer weather, when the temperature of the room rose considerably, that all the tapes showed a systematic shortening. As the series ranged from steel tapes expanding about 1 part in 100,000 per degree Centigrade to invar wires with very small but negative thermal coefficients, it was inferred that there was some error in the thermal equation for the rule given by the International Bureau.

At that time (1917-1918) the best method available for checking this equation was to measure the base in winter (when the room temperature can be easily controlled) at three temperatures and compare with the base one or two tapes of which the thermal expansion coefficients had been determined by other laboratories.

Temperatures of 3°, 17° and 26°C. were used in the tests, each temperature being maintained over a period of three days. The base was measured twice each day and the tapes compared with the base six times. This investigation yielded the equation:—

$$\alpha = (1\ 652\theta + 0\ 00156\theta^2) 10^{-6} \quad (2)$$

The difference between Equations (1) and (2) was of the correct order to account for the discrepancies previously found at the higher temperatures.

However, the method of obtaining Equation (2) was open to the criticism that the exact thermal coefficients of the tapes used were not known to a degree of precision sufficient to place the results beyond doubt, and means were sought to obtain a more reliable value of it.

Later the proposed apparatus for verifying the four-metre rule by means of one-metre standards was installed. An invar tube about 10 cm. diameter was supported by two concrete piers two metres apart, one pier being that carrying the zero bench mark of the base. The tube bears brackets for holding two micrometer microscopes. Until recently it also carried two hangers, for supporting a steel table fitted with two rollers. These rollers are spaced so that they come beneath the Airy points of the one-metre rule, which is placed on them. A screw on each hanger permits the table and rule to be traversed back, out of the field of view of the microscopes. Alternately a



one-metre chord of the four-metre rule can be brought into the position previously occupied by the one-metre rule, using the adjustments on the four-metre rule carriage.

The steps now in operation for obtaining the length of the four-metre rule are:—

(1) Comparison of a nickel one-metre rule with prototypes of the international metre.

(2) Comparison of this nickel rule with one or more invar rules in the rule comparator, both rules under comparison being immersed in water.

(3) Measurement of the four-metre rule, metre by metre, by comparison with an invar one-metre rule. This operation is conducted in air with the aid of the apparatus briefly described above.

The first one-metre rule, No. 191, of invar, was obtained in 1919. At that date the nickel rule had not been received and there was some doubt as to the exact length and the stability of No. 191. Observations made by the Bureau of Standards at different times gave values for the length of No. 191 differing by as much as  $5\ \mu$ . It was not until 1928 that this discrepancy was cleared up, by which time the laboratories at both Ottawa and Washington had been equipped with new comparators for verifying standard rules.

Tests made in the one-metre dilatation comparator of this laboratory confirmed the accuracy of the thermal coefficients of rule No. 191. In this case the coefficients had been determined by the International Bureau from direct observations on the rule itself. Further investigations, in course of which rules and observers were exchanged between Ottawa and Washington and the Ottawa standards were compared directly with the legal prototype metre of the United States, gave concordant results and it was felt that the length of the invar rule No. 191 could be relied upon to within  $0.1$  or  $0.2\ \mu$ .

Furthermore, a new rule, No. 751 of "Fixinvar" (a new alloy said to be less subject to secular change than invar) was obtained. This rule has a thermal expansion of about one-half that of the four-metre rule or No. 191, *i.e.*, about one part in  $10^6$  per degree Centigrade. With two invar rules available, of which the length and thermal coefficients were accurately known, it was considered that a redetermination should be made of the equation of the four-metre rule.

### Investigations of 1932-1933

Previous measurements of the four-metre rule by means of No. 191 had not given such good agreement among themselves as would be expected from the degree of concordance of the actual observations. Therefore before beginning the measurements means were sought for improving their precision.

*Temperature*

The comparisons are conducted in air after the rules have been resting side by side for several hours. In view of the steady temperature of the room it had been assumed that both rules had the same temperature, which was taken as that given by two thermometers on the four-metre rule. Some doubt was cast on this assumption by the fact that during an observation, which lasts about 40 min., the temperature so indicated changed by varying amounts up to 0.5°C. As the rules are of different cross-sectional area it is not likely that both would radiate heat at the same rate.

Accordingly provision was made to mount four thermometers on each rule, the bulbs being protected as far as possible against radiation from objects other than the rules. It was found that the temperatures of the two rules did not change at the same rate nor was the difference between their temperatures constant from test to test. The short rule was found always to have a higher mean temperature by an amount varying from 0.15°C. to 0.45°C. owing, presumably, to the necessary presence of the observers. The test program was therefore altered to provide for the thermometers to be read four or five times during the comparison period.

Table I shows the temperatures recorded by the eight thermometers (after correction for scale errors) during a typical comparison. The readings 1, 2, 3, etc., were taken at approximately five-minute intervals. On the four-metre rule a thermometer was placed near the centre of each metre interval and on the one-metre rule the bulbs of thermometers *F* and *H* were about 5 cm. from the ends of the rule, those of *E* and *G* being near the centre. The higher readings of *F* and *H* were undoubtedly due to the proximity of the observers at the microscopes. In calculating the results of the observations the temperature of each rule was taken as the mean of the indications of the group of four thermometers read during the comparison.

TABLE I

TEMPERATURES RECORDED DURING A MEASUREMENT OF THE FOUR-METRE RULE

Reading No.	Temperature, °C.							
	Four-metre rule				One-metre rule			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>
1	17.20	17.23	17.21	17.27	17.27	17.57	17.27	17.49
2	.25	.29	.27	.28				
3	.30	.34	.32	.33	.52	.76	.56	.68
4	.31	.36	.35	.36				
5	.33	.39	.37	.39	.68	.85	.75	.83
6	.35	.42	.40	.42				
7	.38	.45	.44	.46	.78	18.03	.85	.92
8	.40	.47	.45	.47				
9					.83	.07	.94	.95
	Mean during observation:—17.36°C.				17.73°C.			

### *Rigidity of the Apparatus*

Observations made with the two rules, Nos. 191 and 751, did not agree as well as was anticipated, and a search was made to ascertain the reason. Improved microscopes were first fitted to the apparatus which was readjusted before each set of observations. Soon it became evident that an error was introduced when the one-metre rule and its supporting table were traversed back so that the edge of the four-metre rule could be brought into the field of view of the microscopes. At the time the apparatus was installed it was thought no appreciable error would be introduced from this cause. The torque produced by the movement of the rule is barely detectable by a sensitive level vial and it was concluded that any component of the movement of the microscope axes at  $90^\circ$  from the direction of rule movement would be negligible. A closer study, however, showed that there was a slight error traceable to this cause. The error (perhaps fortunately as it permitted its discovery) could be changed by an appreciable amount, *i.e.*, enough to change the apparent length of the four-metre rule by several microns, by making almost imperceptible changes in the verticality of the microscopes.

Accordingly the rule-supporting hangers were removed from the invar tube, and the one-metre rule table and the traversing slides were carried from a piece of 2 by 4 in. wooden rod attached to the concrete piers. The invar tube became merely a bridge to carry the microscopes. With this change a considerable improvement was found in the concordance of the final results. Despite readjustments of the apparatus the computed length of the four-metre rule remained within a micron of the mean value, which is of the same order as the errors of observation.

### **Determination of the Equation of the Four-metre Rule, 1933**

Observations were made at three temperatures, and on most days the rules were compared three times. The procedure during a comparison was as follows:—microscopes pointed on: (a) one-metre rule; (b) interval 0-1; (c) interval 1-2; (d) one-metre rule; (e) interval 2-3; (f) interval 3-4; (g) one-metre rule; (h) interval 3-4; (i) interval 2-3; (j) one-metre rule; (k) interval 1-2; (l) interval 0-1; (m) one-metre rule.

The temperature of the one-metre rule was recorded between the interchange of observers at *a*, *d*, *g*, *j* and *m*, and that of the four-metre rule was taken between *b* and *c*, *e* and *f*, *h* and *i* and *k* and *l*. A set of observations consumed about 45 min.

The lengths and temperatures of the four-metre rule measured during the tests are given in Tables II and III, which also show the computed lengths of the rule using Equation (5) below, derived as a result of this investigation.

In calculating the equations it was assumed that the expansion of the four-metre rule was linear over the range of temperature covered by each of the three groups of comparisons. The greatest error involved is of the order of  $1\mu$ , and with the exception of one case the errors are much less than this. Hence, with the order of the precision sought, 1 to 2 parts in 4,000,000, the extra labor of a least square reduction was not justified.

TABLE II  
MEASUREMENT OF FOUR-METRE RULE IN TERMS OF RULE 191

Date 1933	Four-metre rule		Four-metre rule, computed (Eq.5)	Observed — computed
	Temp., °C.	Length, 4 m.		
Feb. 7	4.72	$-6.7\mu$	$-6.7\mu$	$0.0\mu$
	4.87	$-5.6$	$-5.6$	0.0
	4.99	$-5.0$	$-4.8$	$-0.2$
Feb. 8	4.72	$-6.3$	$-6.7$	+0.4
	4.93	$-5.3$	$-5.2$	$-0.1$
	5.03	$-2.9$	$-4.5$	+1.6
Feb. 11	4.63	$-7.0$	$-7.3$	+0.3
	4.80	$-5.8$	$-6.1$	+0.3
	4.82	$-5.9$	$-6.0$	+0.1
Feb. 13	4.76	$-6.1$	$-6.4$	+0.3
	4.78	$-6.0$	$-6.2$	+0.2
	4.80	$-5.5$	$-6.1$	+0.6
Feb. 24	17.35	+78.8	+78.2	+0.6
	17.34	+79.3	+78.1	+1.2
	17.33	+79.4	+78.1	+1.3
Mar. 2	28.69	+151.7	+151.1	+0.6
	29.57	+156.7	+156.7	0.0
	30.22	+160.4	+160.7	$-0.3$
Mar. 3	29.36	+153.9	+155.4	-1.5
	29.69	+157.3	+157.4	-0.1
	30.11	+159.2	+160.1	-0.9
Mar. 7	30.13	+160.3	+160.2	+0.1
	30.18	+160.5	+160.5	0.0
	30.31	+161.2	+161.3	-0.1
Mar. 8	30.20	+160.6	+160.6	0.0
	30.48	+163.9	+162.4	+1.5
	30.46	+161.8	+162.2	-0.4
April 24	17.73	+83.1	+80.7	+2.4
	17.89	+82.1	+81.7	+0.4
	17.96	+83.7	+82.2	+1.5
April 25	17.05	+75.6	+76.2	-0.6
	17.27	+79.3	+77.7	+1.6
	17.38	+78.9	+78.4	+0.5

TABLE III  
MEASUREMENT OF FOUR-METRE RULE IN TERMS OF RULE 751

Date 1933	Four-metre rule		Four-metre rule computed (Eq.5)	Observed — computed
	Temp., °C.	Length, 4 m.		
Jan. 25	18.49	+84.8 $\mu$	+85.7 $\mu$	-0.9 $\mu$
	18.57	+84.6	+86.2	-1.6
	18.67	+84.6	+86.8	-2.2
Feb. 3	4.55	-8.7	-7.8	-0.9
	4.85	-6.7	-5.8	-0.9
	5.05	-5.6	-4.4	-1.2
Feb. 6	4.63	-7.5	-7.3	-0.2
	4.77	-6.4	-6.3	-0.1
	4.90	-5.3	-5.4	+0.1
Feb. 9	4.59	-7.3	-7.5	+0.2
	4.75	-6.1	-6.4	+0.3
	4.85	-5.9	-5.8	-0.1
Feb. 10	4.48	-8.9	-8.3	-0.6
	4.67	-7.2	-7.0	-0.2
	4.78	-5.6	-6.2	+0.6
Feb. 23	17.35	+78.2	+78.2	0.0
	17.37	+77.6	+78.3	-0.7
	17.36	+77.4	+78.2	-0.8
Feb. 28	30.28	+161.7	+161.1	+0.6
	30.60	+164.0	+163.1	+0.9
	30.65	+163.5	+163.4	+0.1
Mar. 1	30.23	+161.7	+160.8	+0.9
	30.55	+163.0	+162.8	+0.2
	30.68	+163.6	+163.6	0.0
Mar. 4	30.27	+160.9	+161.1	-0.2
	30.34	+161.7	+161.5	+0.2
	30.32	+161.2	+161.4	-0.2
Mar. 6	30.18	+160.7	+160.5	+0.2
	30.41	+162.5	+161.9	+0.6
	30.36	+161.5	+161.6	-0.1
April 21	17.75	+79.9	+80.8	-0.9
	17.84	+80.6	+81.4	-0.8
	17.90	+81.5	+81.8	-0.3
April 22	17.74	+80.0	+80.7	-0.7
	17.90	+79.8	+81.8	-2.0
	18.00	+81.3	+82.4	-1.1
	18.06	+82.0	+82.8	-0.8

The equations found for the four-metre rule were:—

From the observations with rule No. 191, alone,

$$L_{191} = 4m + (-39.79 + 7.159\theta - 0.0175\theta^2)10^{-6} \quad (3)$$

From the observations with rule No. 751, alone,

$$L_{751} = 4m + (-38.79 + 6.787\theta - 0.0059\theta^2)10^{-6} \quad (4)$$

The mean of Equations (3) and (4) is—

$$L = 4m + (-39.29 + 6.973\theta - 0.0117\theta^2)10^{-6} \quad (5)$$

The difference between Equations (3) and (4) is—

$$L_{191} - L_{751} = (-1.00 + 0.372\theta - 0.0116\theta^2)10^{-6} \quad (6)$$

Equation (6) has a maximum value at 16.04°C. when its value becomes 1.99  $\mu$ , or 1 in 2,000,000 of the length of the rule.

The difference between Equations (1) and (5) is—

$$L_{1.9} - L_{N.R.L} = -0.021\theta - 0.0086\theta^2 \quad (7)$$

Equation (7) gives a value of about  $8.1 \mu$  or 1 part in 500,000 at  $30^{\circ}\text{C}$ . This is the order of the shortening found in the laboratory standard tapes verified at this temperature when their lengths were computed from the equation of the four-metre rule given by the International Bureau of Weights and Measures.

### The One-metre Rules

*Rule No. 191.* Invar rule 191 is of an inverted T-section, with graduations ruled to the edge of the exposed neutral surface. One edge is graduated throughout in millimetres from 0 to 1000, with an extra millimetre beyond each end of this range subdivided to 0.1 mm. The thermal expansion determined by the International Bureau of Weights and Measures is

$$\alpha = (2.117\theta - 0.00503\theta^2)10^{-6}$$

Rule No. 191 bears the inscription "Coulée 1209" which is also stamped on the four-metre rule. Hence it would appear that it furnishes further evidence that different samples of invar from the same ingot will not have identical thermal coefficients.

*Rule No. 751.* Fixinvar rule No. 751 is of orthodox H-section, with graduations, similar to those on No. 191, ruled along the axis on the neutral surface. The thermal equation determined by the International Bureau of Weights and Measures is

$$\alpha = (0.997\theta + 0.00402\theta^2)10^{-6}$$

### Thermometers

The temperatures of the rules were measured by Poulenc mercury thermometers subdivided to  $0.1^{\circ}\text{C}$ . Their corrections in the horizontal position were determined by comparison with the standard thermometers of the Division.

### Reference

1. TOPOGRAPHICAL SURVEY OF CANADA. Department of the Interior Ottawa. Bull. 44. 1921.

## ON THE THERMAL CONDUCTIVITY OF VARIOUS INSULATORS AT ROOM TEMPERATURE<sup>1</sup>

BY C. D. NIVEN<sup>2</sup>

### Abstract

The values for thermal conductivity of various common materials chiefly used in the walls of houses are given. By plotting the results obtained, as well as those obtained by other experimenters, on a density-conductivity diagram there is a general indication that at higher densities thermal conductivity increases with increase of density much more rapidly than it does at low densities.

### Introduction

The thermal conductivity of many of the commoner materials has become a matter of some economic importance ever since the public have appreciated the fact that by insulating the walls of houses, substantial savings in fuel consumption can be effected. To ascertain the efficiencies of the various thermal insulators a hot plate was erected at the National Research Laboratories at Ottawa about two years ago; the description of this apparatus has already been published in this journal (3) together with an account of an extensive investigation on the thermal conductivity of fibre board. That work was undertaken largely with a view to ascertaining the various factors involved in carrying out accurate measurements on a hot-plate apparatus.

In this communication, the data on other materials are set out and a general comparison is made of various substances. In order to avoid comparisons between the products of the makers and sellers of these materials it has been thought wise to omit the trade names. The scientific value of the results is not in any way impaired thereby. In order not to confuse the reader by the mere use of numbers the materials have been classified to some extent. Boards have been designated by the letter *B*, soft materials by the letter *S*, fillers by the letter *F*, and some miscellaneous materials by the letter *M*; a single descriptive word usually has been given in brackets. This may serve to remind the reader, when reading the table of values, of the full description of the material given in the text. As the data are of economic interest the words "Canadian" or "imported" have been added, to indicate whether or not the insulator is a Canadian product.

### Experimental Data

The units in which the results are expressed are as follows:—Thickness in inches: moisture in %: density in pounds per cubic foot: mean temperature in degrees Fahrenheit: conductivity in B.T.U. per hour, per square foot, per degree Fahrenheit, per inch. The results are presented in Table I. Values

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which have been already reported by the writer (3) have not been repeated; reference, however, has been made to them in the text. The descriptions of the materials measured are as follows.

*B 1 (Wood-fibre)—Canadian.* This board is made from wood-fibre; four different thicknesses or kinds have been tested, namely one-inch, half-inch, one-inch low density and two-inch. For results see Ref. 3, pp. 123-125. It is there referred to as Wood Fibre No. 1. The results on a two-inch sample, not reported at that time, are given in Table I.

*B 2 (Wood-fibre)—Canadian.* This is a board somewhat similar to *B 1*; the fibre is to all appearances slightly larger. Two thicknesses were measured, namely one-inch and half-inch. For results see Ref. 3, pp. 123-125. This board is there referred to as Wood Fibre No. 2.

*B 3 (Bagasse)—Imported.* This is a board made from sugar-cane waste. The fibres in it are much longer than those in either of the wood-fibre boards referred to already. For results see Ref. 3, pp. 123-125.

*B 4 (Wood-fibre)—Canadian.* This board is somewhat similar in appearance to *B 2*. A half-inch sample was measured.

*B 5 (Laminated)—Canadian.* This board consists of five pieces of soft cardboard-like material stuck together with asphalt.

*B 6 (Cardboard)—Canadian and Imported.* This looks like a very thick rigid cardboard; it is more of a house lining than an insulator.

The first two results in Table I refer to a different make of board from that to which the last three refer. The boards, however, were so similar that they have all been grouped as *B 6*.

*B 7 (Cork)—Imported.* Only one-inch samples were measured.

*B 8 (Plaster Board)—Canadian.* This board consists of gypsum plaster between two sheets of paper. It has a perfectly smooth hard finish and is fairly strong but can hardly rank as an insulator as its thermal conductivity is high.

*B 9 (Plaster Board)—Imported.* Both samples of this board were much thinner than *B 8*, although otherwise similar in appearance.

*S 1 (Flax Waste)—Canadian and Imported.* This material is made from flax waste and comes in sheets about  $\frac{3}{4}$  in. thick. It resembles a mat but is not strong and requires to be supported in position.

*S 2 (Eel Grass)—Canadian and Imported.* This consists of eel grass packed between two sheets of brown paper and held in place by sewing right through.

*S 3—Imported.* This is a woolly material from wool sewn between sheets of brown paper somewhat like *S 2*.

*S 4 (Animal Wool)—Canadian.* This board consists of wool sewn between sheets of paper.

*S 5 (Sheep's Wool)—Canadian.* Sheep's wool as used for clothing sewn between two sheets of thick paper; the product is much thicker than *S 4*.



*F 1 (Plaster Board Trimmings)—Canadian.* This is a composite mixture of gypsum and paper.

*F 2 (Asbestos Cement).* This is the common asbestos cement powder.

*F 3 (Sawdust).* Ordinary sawdust, and sawdust containing 13.6% calcium chloride.

*F 4 (Fibre Board Trimmings)—Canadian.* This consists of trimmings off the fibre board *B 2*.

*F 5 (Gypsum).* This is a gypsum product which, when mixed with water and poured into a mold, sets as a porous mass making a good insulator, provided the manufacturer's instructions are properly carried out. In practice it is found that this is not always the case.

*F 6 (Asbestos and Crushed Rock)—Canadian.* Three samples of mixtures of asbestos and crushed rock were tested.

*M 1 (Lime and Diatomite)—Canadian.* Three samples consisting of lime and diatomite (percentages not known) were tested.

*M 2 (Aluminium Foil, Spaced).* A sample, consisting of three sheets of aluminium foil and two sheets of paper stretched on a frame about one inch thick, was tested. The three aluminium sheets were inside and the two sheets of paper on the outside. They were spaced to give four equal air spaces.

*M 3 (Paper, Spaced).* A sample somewhat similar to *M 3*, made of four sheets of common blue-print paper stretched on a frame about one inch thick, was tested.

*M 4 (Concrete and Cinder)—Canadian.* A thick slab of concrete and cinder was tested. The results are not of great value because the moisture could not be accurately ascertained.

TABLE I  
SUMMARY OF RESULTS

Material	Thick- ness, in.	Moist- ure, %	Density, lb. per cu. ft.	Mean temp. °F.	Con- ductivity, B.T.U. per hr., per sq. ft., per °F., per in.
<i>B. 1.</i> (wood-fibre) Canadian	2.128	6.9	19.1	54	0.46
<i>B. 4.</i> (wood-fibre) Canadian	0.520	4.3	16.0	52	0.358
<i>B. 5.</i> (laminated) Canadian	0.471	6.5	20.7	43	0.346
	0.474	6.5	20.6	46	0.340
	0.434	5.9	24.5	45	0.390
	0.433	5.9	24.4	50	0.391
<i>B. 6.</i> (cardboard) Canadian and Imported	0.199	7.8	31.8	54	0.449
	0.198	7.8	31.9	46	0.449
	0.178	6.6	34.8	49	0.481
	0.182	6.6	34.9	48	0.487
	0.182	6.6	34.9	54	0.486

TABLE I  
SUMMARY OF RESULTS

Material	Thick- ness, in.	Moist- ure, %	Density, lb. per cu. ft.	Mean temp. °F.	Con- ductivity, B.T.U. per hr., per sq. ft., per °F., per in.
<i>B. 7. (cork)</i>	0.998	1.56	9.6	46	0.280
Imported	1.029	0.9	11.5	46	0.302
	0.996	1.9	11.3	47	0.313
	0.997	1.4	10.6	45	0.297
<i>B. 8. (plaster board)</i>	0.379	8	48.8	31	0.998
Canadian	0.381	5.6	48.4	29	1.06
	0.507	10	52	54	1.15
containing particles of zonolite	0.524	11	54	53	1.23
containing particles of wood	0.497	10.5	49	53	0.99
<i>B. 9. (plaster board)</i>	0.379	—	57.5	36	0.936
Imported	0.235	—	57.9	35	0.726
<i>S. 1. (flax waste)</i>	0.833	6.4	10.8	50	0.323
Canadian and Imported	0.754	6.4	10.8	49	0.334
<i>S. 2. (eel-grass)</i>	0.228	9.7	6.5	46	0.251
Canadian and Imported	0.222	9.0	6.6	46	0.229
<i>S. 3. Imported</i>	0.397	6.6	6.0	55	0.262
	0.403	6.6	5.8	53	0.267
<i>S. 4. (animal wool)</i>	0.273	8.1	7.9	51	0.229
Canadian	0.270	7.5	7.8	49	0.243
<i>S. 5. (sheep's wool)</i>	0.502	6.7	8.7	53	0.244
Canadian	0.430	8.8	6.8	59	0.235
	0.483	7.8	9.2	53	0.242
<i>F. 1. (plaster board trimmings)</i>	1.174	6.3	32	49	0.610
Canadian					
<i>F. 2. (asbestos cement)</i>	1.130	1.0	45	44	1.1
<i>F. 3. (saw dust)</i>	1.202	8	8.7	48	0.373
with 13% CaCl <sub>2</sub>	1.177	12.4	10.9	45	0.377
<i>F. 4. (fibre-board trimmings)</i>	1.099	5	5.0	43	0.304
Canadian	1.075	5	3.1	44	0.284
	1.092	5	7.3	47	0.298
<i>F. 5. (gypsum)</i>	1.021	0.9	11	46	0.357
<i>F. 6. (asbestos and crushed rock)</i>	1.079	0.5	80	65	1.48
Canadian	1.079	0.6	74	66	1.32
	1.079	0.6	71	64	1.31
<i>M. 1. (lime and diatomite)</i>	1.341	2	42	56	0.87
Canadian	1.281	Less than 1	80	45	1.86
	1.409	1.6	101	48	2.92
<i>M. 2. (aluminium foil, spaced)</i>	1.248	—	—	55	0.253
<i>M. 3. (paper, spaced)</i>	1.095	—	—	45	0.392
<i>M. 4. (concrete and cinder) Canadian</i>	2.690	—	48.5	58	1.28

### Comparison of Results

The thermal conductivity of any substance is generally affected by the mean temperature of the sample and by its moisture content; in certain cases, the duration of test may actually affect the results. But on the assumption, that these factors are small in comparing the results, some rather interesting

conclusions can be drawn regarding the relation between density and thermal conductivity.

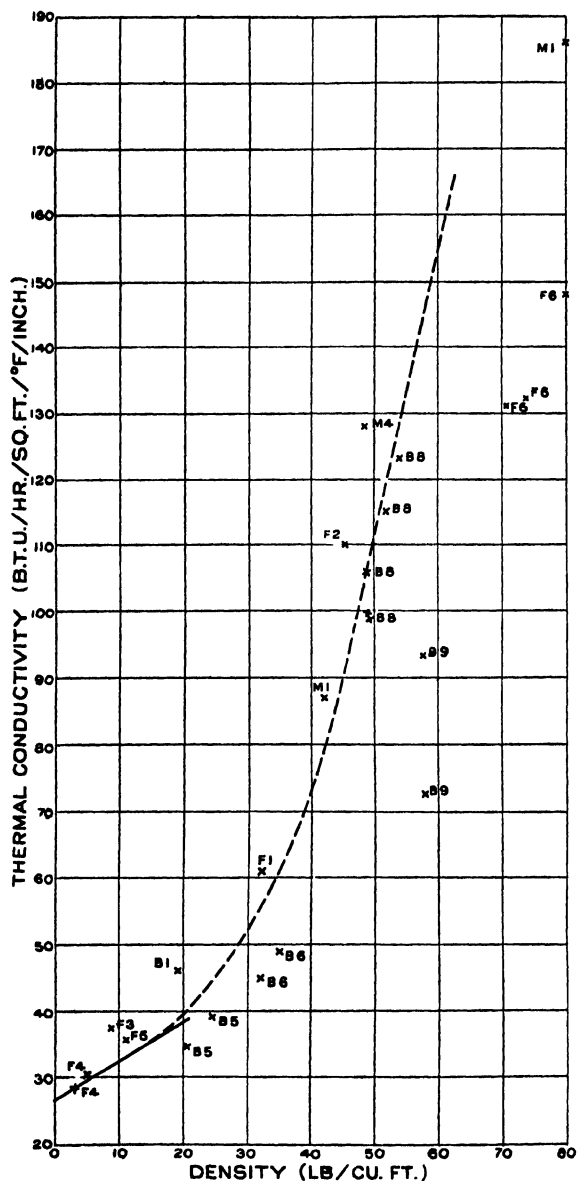


FIG. 1. Graph showing that when the thermal conductivities of the materials tested are plotted against the densities, the points are grouped roughly about a curve.

If the conductivities for one-inch and half-inch samples of fibre board be plotted against the densities, it has been shown in the paper previously published that the points lie approximately on a straight line. If the results on a two-inch sample of B 1 be plotted on such a diagram, it is found that the point lies considerably off the line; in fact, the conductivity of the two-inch board is very much higher than might be expected from the linear relation. Possibly this is due to the arrangement of the fibres. As mentioned in the previous paper, Finck (2) has proved that when the fibres of a material lie across the direction of heat flow the material has greater heat resistance than when they lie along that direction, and strange to say, this point lies nearly on the extrapolated curve through the points plotted from Finck's results on bagasse in the bone-dry condition. Finck's results, as well as the results obtained by other experimenters on fibre board, have been plotted by the writer and it has been found that they lie around the straight-line

relation connecting density and conductivity for dry material. The diagram has not been reproduced in this paper because it was necessary to apply corrections to some of the actual results of the experimenters in order to make their results apply to a mean temperature of 90°F. and a moisture content of 0%. The diagram, however, pointed to the conclusion that the straight line gives an average value for sheets made from vegetable fibres, and on this assumption one should therefore expect that the results on S 1 (flax waste) and S 3 should also lie on this line. By actually plotting these results, it becomes clear that S 1 does lie near the line but not S 3. If the results for S 2 (eel grass) be plotted on this diagram the point lies still farther off; this is perhaps to be expected because the fibre is long and flat and lies across the direction of heat flow. The results on S 4 (animal wool) and S 5 (sheep's wool), when plotted, indicate that animal wool fibres are better insulators than vegetable fibres. The material B 5 is laminated and its conductivity is lower than one would expect for a fibre board of similar density. The results on B 6 (card-board) have been plotted on this diagram but as the densities are much higher than those of the fibre boards it is unsafe to draw any conclusions, especially in view of the results on materials of higher density.

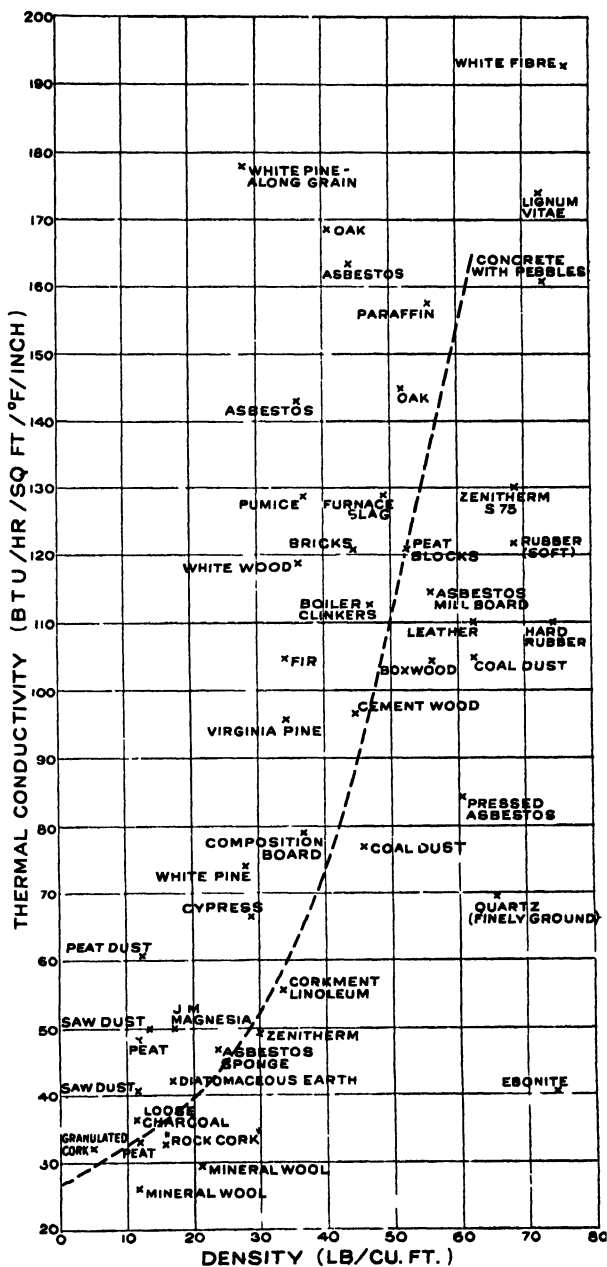


FIG. 2. Graph showing results taken from A.S.R.E circular (1) in relation to curve in Fig. 1.

It has been found that when the results on materials of higher density are plotted, the points thus obtained lie very roughly on a curve. The points are plotted on Fig. 1. In Fig. 2 this curve, on which the points roughly lie, is reproduced and the results on a number of substances are plotted; these results have been taken from the tables at the end of a report by the A.S.R.E. insulation committee (1).

The line near the origin on Fig. 1 is the line obtained for fibre boards. It becomes now very obvious that although *B* 6 does lie on this straight line when produced, we have no right to conclude that a straight-line relation holds between density and conductivity for vegetable fibres with a density greater than 20.

It is not the purpose of this paper to discuss the results plotted in Fig. 2; that diagram serves, however, to confirm the conclusion which may be drawn from Fig. 1, namely, that in general, at higher densities increase in density causes a much greater increase in thermal conductivity than it does at lower densities.

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## THE SORPTION OF WATER BY ASBESTOS FIBRE<sup>1</sup>

BY L. M. PIDGEON<sup>2</sup> AND A. VAN WINSEN<sup>3</sup>

### Abstract

The sorption of water by asbestos fibre has been examined using two modifications of the static method. In the first method the sample was weighed in the absence of air by a quartz spring balance, after which the required vapor pressure of water was admitted. In the second method the samples were weighed in air in a room the temperature and humidity of which were controlled. The two methods have given identical results, producing the sigmoid isotherm similar to those found in the cases of cotton and wood, and exhibiting a marked inflexion in the region of the saturation point. The rate of sorption has been examined using the sorption balance and it has been shown that at humidities below 70% equilibrium is established in a few minutes; at higher humidities longer time is required. Because of this and other reasons it is suggested that in this region capillary condensation is taking place.

### Introduction

It would appear that the sorption of water should be an important factor influencing the physical properties of a fibrous material like asbestos, which retains its special nature due to the water of hydration. Research carried out in these laboratories on the standardization of asbestos testing has shown this to be the case, as the relative humidity appears to exert a definite effect on the screen-test results. This variation in the screen test must be dependent on the amount of sorbed water which in turn is controlled by relative humidity. The obvious starting point in an examination of this factor is a determination of the amount of sorption under different conditions of humidity.

Very few references to quantitative work of sorption by asbestos are available and in most cases the type of fibre and its previous history are not mentioned. Wilson and Fuwa (4) gave a few values for asbestos in a survey of the moisture relations of a large number of substances, but no details of the fibre are given. It seemed desirable to obtain data on the moisture equilibria of the fibre as it is used in the Quebec standard testing machine. This paper describes experiments carried out by two different methods to find the amount of sorption over a complete range of humidities from 0 to 96%.

### Samples

The samples which were examined were from a standard, milled product of the chrysotile variety, obtained in the province of Quebec. No washing or other treatment was given. The screen test of the fibre was approximately 4-8-4 Quebec Standard Test.

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## Experimental

The sorption of water was examined by two methods which differ in the procedure by which the required vapor pressure of water was obtained.

### Method 1.

A sorption balance was employed, sorption taking place in the absence of air. The apparatus (Fig. 1) was a modification of the arrangement described by McBain and Bakr (2), which has been employed by one of the authors

elsewhere (3). The sample was suspended by a fine aluminium wire from a quartz spiral in the chamber *C*. The water was contained in the tube *B* and was freed from dissolved gases in the ordinary manner. The pressure in the apparatus was measured on the manometer *A*, which was filled with an oil of negligible vapor pressure, giving a deflection 15 times that of mercury. The system was evacuated with a two-stage steel mercury diffusion pump backed by an oil pump, with which combination the pressure could be reduced to less than  $10^{-5}$  mm. Hg. The tube containing the quartz spiral was immersed in a thermostat filled with water which was maintained at  $20^{\circ}\text{C.} \pm .01^{\circ}$ .

A deflection of 1 mm. was produced by a weight of 0.0075 gm., with the cathetometer reading to 0.02 mm.; a change of weight equal to 0.0002 gm.

could therefore be detected. Hence with a sample weighing 0.3 gm. the measurements of weight were correct to 0.1%.

### Procedure

Two methods of operation are available with this apparatus; they may be called the constant vapor pressure method and the variable vapor pressure method. In the first, the vapor pressure of the apparatus is maintained at a constant value by controlling the temperature of the bulb *B*. This method requires a second thermostat to attain this end. The great advantage is that the sorption takes place at a constant vapor pressure throughout the whole period. In the second method, a certain amount of water vapor is added to the apparatus by momentarily opening the tap over tube *B*. As adsorption takes place the pressure falls until equilibrium is established. This method, while it is much more convenient than the first, is unsuitable if hysteresis effects are to be examined, as adsorption takes place during a falling

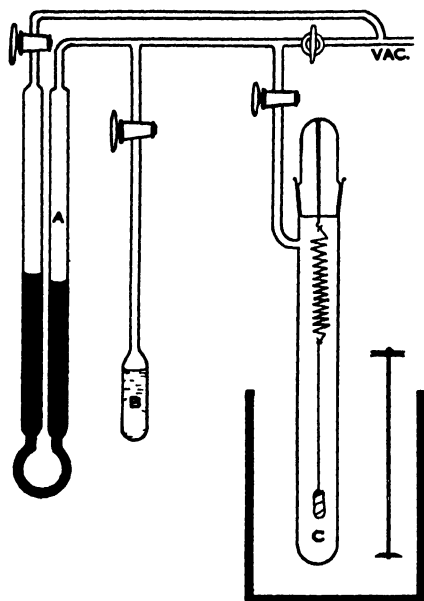


FIG. 1. Sorption balance.

vapor pressure, hence some desorption takes place simultaneously. In the present case it was found that the value at any humidity was independent of the direction in which the equilibrium was reached, hence the second method was generally adopted for its convenience.

### *Method 2*

In the second series of experiments the samples were exposed to air of known humidity and temperature in a conditioning room. The temperature was controlled to  $0.5^{\circ}\text{C}$ . by means of a bimetallic thermoregulator. The air in the room was circulated with a fan. The relative humidity was maintained by a mechanical sprayer controlled by a membrane-type humidostat. The humidity was maintained to within 2% of any stated value up to 60% relative humidity, while above this value the variations became more noticeable. The humidostat was periodically checked against an aspiration psychrometer.

Samples (500 gm.) were exposed in this room in such a way as to be protected from any mist or spray. This weight was chosen to satisfy the requirements of the Quebec standard testing machine which requires one-pound samples. Forty eight hours was allowed for the establishment of equilibrium after which the samples were weighed to 0.1 gm. giving an accuracy of weight determination of 0.02%.

In comparing the two methods it may be stated that while the weight determination is much more accurate in the case of Method 2, owing to the large size of the samples which may be employed, the determination of relative humidity is liable to be seriously in error at high values. In the first method the humidity determinations are dependent only on the measurement of temperature and pressure, both of which may be accurately determined. The results in the case of the sorption balance are considered to be more accurate in the region of high humidities, while at lower humidities agreement is almost perfect as will be seen later.

The sorption balance offers additional advantages in that the conditions may be accurately controlled and a wider range of humidities produced, while the time required for the establishment of equilibrium is enormously reduced by the removal of air. The second method is important, however, as it is identical with the conditions which are encountered in practice.

## **Experimental Results**

### *Definition of "Dry Weight"*

It is necessary to define the "dry" weight of all hydrated compounds, as by heating to elevated temperatures it is always possible to drive off more water and the values at any humidity will obviously depend on the dryness which has been chosen as the zero value. It is undesirable to heat asbestos to high temperatures because of the danger of removing part of the water of hydration; hence in the experiments to be described the following procedure was adopted.



In the vacuum method as described above the dry point was obtained by strongly evacuating the sample at 20°C. A constant reproducible weight was reached after a few hours, evacuation upon which the subsequent calculations were based. For Method 2 the dry point was obtained by heating the sample in air at 105°C. for 16 hr. The data to be given show that these methods give identical results. It follows, therefore, that heating to 105°C. has no effect on the chemically bound water or on the power of adsorption.

TABLE I  
SORPTION OF WATER BY ASBESTOS FIBRE

Method 1 Temperature 20°C.		Method 2 Temperature 25.5°C.	
Relative humidity, %	Sorption, %	Relative humidity, %	Sorption, %
6.5	0.5	24	0.82
11.3	0.7	33	0.97
27.6	0.9	40	1.09
49.5	1.2	50	1.16
63.0	1.4	60	1.32
68.0	1.5	70	1.57
78.7	1.9	80	2.10
85.6	2.2	90	2.53
96.6	3.3	96	3.14

The results shown in Table I are plotted in Fig. 2 and show good agreement between the two methods except at high humidities. It may be safely assumed that the results by Method 1 will be more nearly correct above 75% relative humidity. The curve is of the familiar sigmoid shape which characterizes many sorption processes. Three definite regions are in evidence; at low humidities (up to 5%)

adsorption takes place at very low pressure causing the curve to rise sharply; then follows a wide region from 20 to 65% in which the isotherm approaches a straight line of definite slope; near the saturation point it again rises sharply giving a relatively high and rather indefinite value for the saturation point. Values as high as 6% have been obtained by allowing the fibre to remain in a saturated atmosphere for some time. The significance of the isotherm will be discussed later.

#### Rate of Adsorption

The sorption balance offers a convenient method of determining the rate

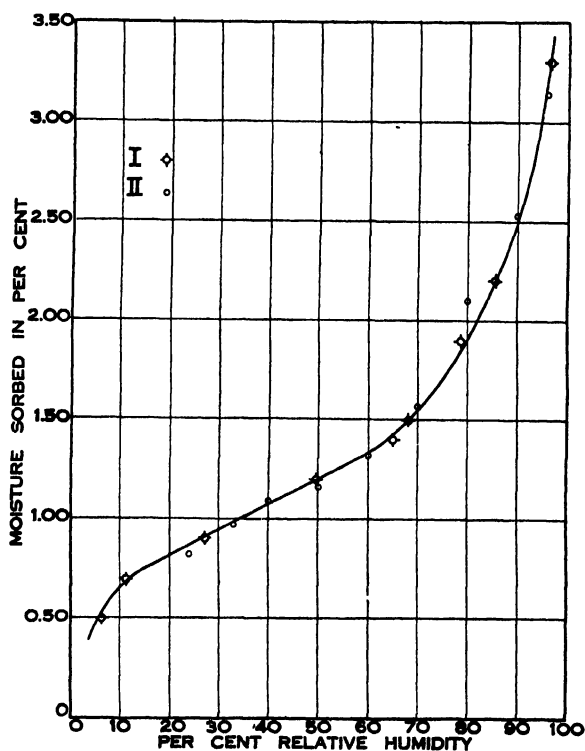


FIG. 2. Sorption of water by asbestos.

at which adsorption takes place in the absence of air. Under these circumstances, the true rate of adsorption is more closely approached as the molecules of adsorbate are not required to diffuse through inert molecules to reach the adsorbing surface. Any adsorption establishes a pressure gradient which is quickly removed by mass flow of the gas. The procedure which was followed included the evacuation of the sample in the usual manner, after which the tube *B* was placed in communication with the apparatus, the temperature of the water in *B* being controlled to produce the desired vapor pressure. Readings of deflection were taken against time. There was some delay in the establishment of the final vapor pressure owing to the cooling of the water surface by evaporation. This did not appear to be a serious objection as the experiments on rate are largely comparative.

The results of experiments performed at different humidities are plotted in Fig. 3. It is at once apparent that at humidities corresponding to the straight part of the isotherm the equilibrium is established in a few minutes, suggesting a true surface adsorption in the ordinary sense of the term. This rapid adsorption process holds over the straight part of the isotherm, while

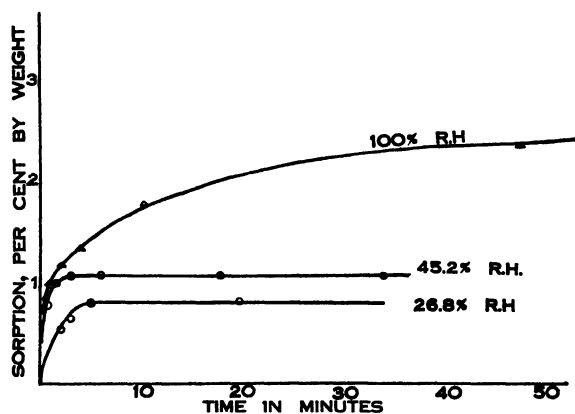


FIG. 3. Rate of sorption.

at high humidities, where the amounts taken up are large, a further process appears to be superimposed. Much longer times are required, the equilibrium being incomplete after the lapse of several hours. The theoretical significance of this will be mentioned below.

### Hysteresis

In the cases of silica gel, aluminium hydroxide, ferric oxide, and other porous inorganic substances, considerable controversy has arisen as to whether the same sorption value at a given humidity may be reached either by gain of water from a less saturated condition or by loss from a higher saturation. This point has been examined for asbestos using the sorption balance. The apparatus was altered by the addition of a one-litre flask containing a sulphuric acid solution, the concentration of which was adjusted to give the desired humidity. A very constant source of relative humidity was obtained by immersing the flask, equipped with a magnetic stirrer, in the thermostat.

At relative humidities of 30.0, 45.0 and 79.0% the same sorption value was reached by adsorption or desorption; hence no hysteresis exists in the case of asbestos fibre over this range. The experimental significance of this fact has been already mentioned.

## Discussion

The sorption isotherms for water on asbestos suggest a direct adsorption process which is rapidly completed up to humidities of 70% of saturation. Above 70% the sorption process takes on a new form; not only is the amount of sorption much greater but the time taken to reach equilibrium is a great deal longer. If the results are plotted logarithmically according to the classical sorption isotherm

$$x/m = kp^{1/n}$$

where  $x/m$  is the amount of sorption,  $k$  and  $n$  are constants, and  $p$  the pressure, a straight line is obtained for values extending from 5 to 65% of saturation; as shown in Fig. 4. Above the latter value the curve bends sharply toward the sorption axis again suggesting some change in the mechanism of sorption.

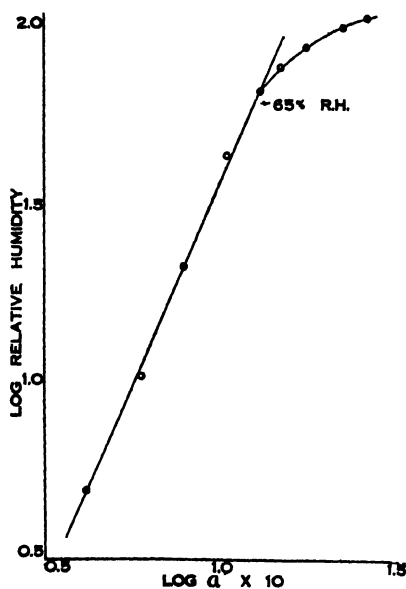


FIG. 4. Sorption of water by asbestos.

It is suggested that this upper region is due to condensation of liquid in capillaries. While the evidence for capillary condensation as a general explanation for sorption may not be completely convincing it is generally admitted that in certain instances, where the isotherm is of the shape found here, capillary condensation takes place at high humidities. It is mentioned by McBain (1) that various hydrated oxides (copper, manganese, etc.) show an isotherm which gives evidence of capillary condensation near the saturation point; hence it appears that asbestos may be added to their number.

As mentioned previously the practical importance of this subject lies in its application to standardization of asbestos testing. It is of particular interest, therefore, to have found that the sorption isotherm between relative humidities of 25 and 65% approaches a straight line. If a direct relation may be found between the amount of sorbed water and the physical condition of the fibre as shown by the screen test, the test result at any other humidity should be predictable from one determination together with consideration of the sorption isotherm.

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# THE ACTION OF SULPHURIC ACID ON CERTAIN DERIVATIVES OF CYCLOPROPANE<sup>1</sup>

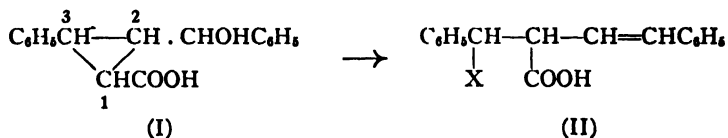
BY CHARLES F. H. ALLEN<sup>2</sup> AND RAYMOND BOYER<sup>3</sup>

## Abstract

A considerable variety of ketocyclopropanes has been submitted to the action of sulphuric acid, alone or in acetic acid. In general, the mode of reaction was similar to, though less drastic than, that of hydrogen bromide; this indicated a similar mechanism. When the cyclopropane ring was attacked, the product isolated was apparently formed by the addition of a molecule of water or acetic acid, or was the result of a secondary reaction dependent on a primary product so formed. The nitriles were hydrolyzed to amides without opening of the ring. A mechanism is suggested to account for the ring scission of cyclopropyl alcohols, which takes place in a different manner from most cyclopropane derivatives.

Kohler and his students have studied in great detail a large number of highly substituted cyclopropane ketones and determined characteristic reactions of each type. The similarity to  $\alpha$ ,  $\beta$ -unsaturated ketones has been pointed out; in addition reactions involving substances of the type HX, the X was always attached to a  $\beta$ -carbon, just as in 1,4-addition to a conjugated system. As far as addition was concerned the cyclopropane ring was equivalent to an ethylenic linkage (17). This same conclusion has been reached by other investigators; *e.g.*, from a study of absorption spectra Carr and Burt (9) concluded that the cyclopropane ring is very similar in character to an ethylenic linkage and can, therefore, form a conjugated system with a carbonyl group.

The only discrepancy appears in some work of Stoermer (23) in which the action of sulphuric acid, alone or in acetic acid, on the hydroxyacid (I) was shown to give an open chain substance of the type of (II); this could have been formed only by opening of the ring in the 2 : 3-position.



The purpose of this investigation was to see whether sulphuric acid acted in a different manner from other ring-splitting reagents.

Sulphuric acid was found to be less reactive than hydrogen bromide as would be expected, but the mode of reaction was in general the same. When the cyclopropane ring was attacked the product isolated was apparently formed by the addition of a molecule of water or acetic acid, or was the result of a secondary reaction dependent on a primary product so formed. The reagent was an acetic acid solution of sulphuric acid, the concentration

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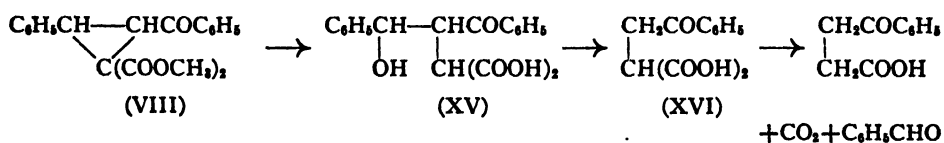
being varied according to the ease with which the substance reacted\*. If the starting material was recovered unchanged, cold concentrated sulphuric acid alone was used. The results are shown in Table I. The positions are numbered in the usual manner (cf. formula I).

TABLE I  
ACTION OF SULPHURIC ACID ON CYCLOPROPANES

No.	Substituents in position			Result	Ring opening	
	1	2	3		by H <sub>2</sub> SO <sub>4</sub>	by HBr
III	2 H	C <sub>6</sub> H <sub>5</sub> CO H	2 H	γ-Acetoxybutyro- phenone	1,2 or 2,3	1,2 or 2,3 (22)
IV	C <sub>6</sub> H <sub>5</sub> C <sub>6</sub> H <sub>5</sub> CO	C <sub>6</sub> H <sub>5</sub> CO H	2 H	Unattacked (1)	No	1,2 (1)
V	C <sub>6</sub> H <sub>5</sub> CO H	C <sub>6</sub> H <sub>5</sub> CO H	C <sub>6</sub> H <sub>5</sub> H	Bimolecular product	Undetermined	1,3 or 2,3 (18) Monomolecular
VI	2 COOR or 2 COOH	C <sub>6</sub> H <sub>5</sub> CO H	2 H	Unattacked (3)	No	1,2 (3)
VII	COOH H	C <sub>6</sub> H <sub>5</sub> CO H	2 H	Unattacked (3)	No	—
VIII	2 COOCH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub> CO H	C <sub>6</sub> H <sub>5</sub> H	C <sub>6</sub> H <sub>5</sub> CHO C <sub>6</sub> H <sub>5</sub> COCH <sub>2</sub> CH <sub>2</sub> COOH	1,3 2,3 not excluded	1,3 and 2,3
IX	C <sub>6</sub> H <sub>5</sub> COOCH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub> CO H	C <sub>6</sub> H <sub>5</sub> H	Unattacked	No	1,2†
X	H NO <sub>2</sub>	p-ClC <sub>6</sub> H <sub>4</sub> CO H	C <sub>6</sub> H <sub>5</sub> H	Furane (XV)	2,3	2,3 (19)
XI	C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	C <sub>6</sub> H <sub>5</sub> CO H	2 H	Unattacked until concd., then oils	Undetermined	No
XII	2 H	CN COOR	2 H	Amide ester (XIX)	No	—
XIII	C <sub>6</sub> H <sub>5</sub> CN	C <sub>6</sub> H <sub>5</sub> CO H	C <sub>6</sub> H <sub>5</sub> H	Amide (XXIII)	No	No
XIV	2 H	H (C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub> COH	2 H	Oils	Undetermined	—

† Unpublished results.

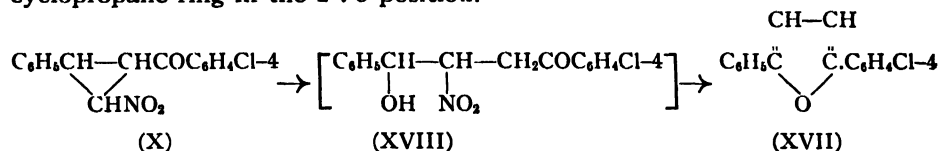
The ester (VIII) gave as final products benzaldehyde and β-benzoyl propionic acid. Two intermediates of known structure (XV, XVI) were isolated, so that the probable course of the reaction is as follows:



\*Bertram and Walbaum (4) used such a mixture for the hydration of camphene, and it has been applied similarly to other terpenes.

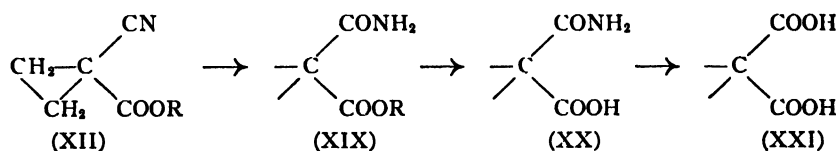
The ring has apparently been opened in the 1 : 3 position; however, the amount of acid (XV) was not sufficient to exclude 2 : 3 ring opening, which would also yield benzaldehyde and acid (XVI).

The nitroketone (X) was very easily attacked and gave 2-phenyl-5 (*p*-chlorophenyl) furane (XVII), resembling the behavior of the same substance with hydrogen bromide (1); it was impossible to isolate an intermediate product, but by analogy with the known reaction it should be a hydroxy compound (XVIII). At any rate, the net result has been a splitting of the cyclopropane ring in the 2 : 3 position.

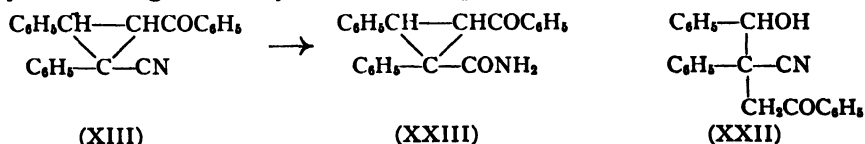


Diels has shown that furane and its methylated homologues react in the diene synthesis, forming addition products with maleic anhydride (11). This addition is apparently blocked by the presence of phenyl groups on the  $\alpha$ -carbon, for the furane (XVII), 2,5-diphenylfuran and 2,3,5-triphenyl-4-bromofuran were recovered unchanged after refluxing with maleic anhydride.

The cyclopropane nitriles all reacted alike, being hydrolyzed to amides without ring opening. The nitrile ester (XII) gave the amide (XIX) which was then hydrolyzed to the known dibasic acid (XXI) by alkali, thus showing the ring was still intact. The intermediate acid-amide (XX) was isolated.



All three stereoisomeric forms of the cyanoketone (XIII) (16) gave the same substance, analyzing for the addition of water and different from the two known open chain stereoisomers (XXII) (16). The new substance is the amide (XXIII); it could not be hydrolyzed by any method tried. On dehydration it gave the cyclic nitrile, m.p. 166° C.



A very small amount of a stereoisomeric amide was formed from one of the cyanoketones, as well as being the sole product of the action of ammonia on the ester (IX). It was converted into its isomer by the action of alcoholic hydrochloric acid; the reverse transformation was brought about by acetic anhydride. Cyclopropyl cyanide and 1-phenyl-1-cyanocyclopropane have already been hydrolyzed to amides without opening of the ring by the action of alkali or phosphoric acid (7, 14) and were not included in this work.

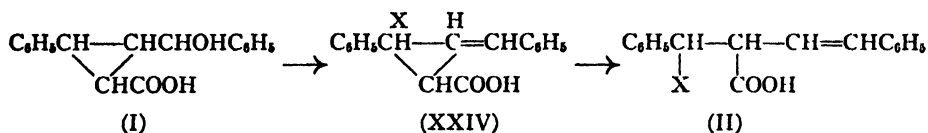
From these results it is evident that there is nothing peculiar about sulphuric acid as a ring-opening reagent; therefore to account for the results described by Stoermer with the hydroxycyclopropane (I) some other mechanism must be devised, especially as hydrogen chloride acted in the same manner to give a chloride (formula II; X-Cl). The following generalizations are suggested:

1. When cyclopropane alcohols in which the OH is on the carbon next the ring are treated with acidic reagents that open the ring, the first step is a dehydration to form a substance having a double bond directly connected to the ring.

2. The system thus formed acts as the equivalent of an allenic linkage and, in addition reactions, adds reagents of the type HX like a highly substituted allene, the X going to an end carbon and the H to the central carbon, with splitting of the ring.

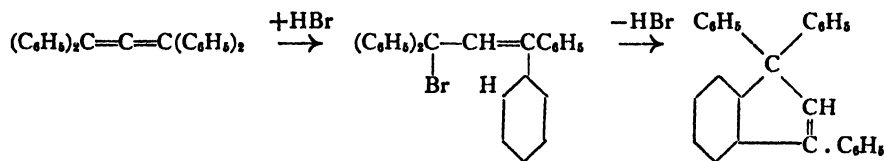
3. When both an alcoholic and carboxyl group are attached to the ring, the former system is more active.

These may be illustrated with substance (I). Stoermer (23) suggested that (XXIV) might be an intermediate product in the reaction, though no com-



pound with such a linkage was then known. In a recent attempt to make cyclopropanone, Lipp (20) converted diphenylcyclopropylcarbinol (XIV) to the bromide and removed hydrogen bromide to get (XXV) showing that a substance of this type is capable of existence.

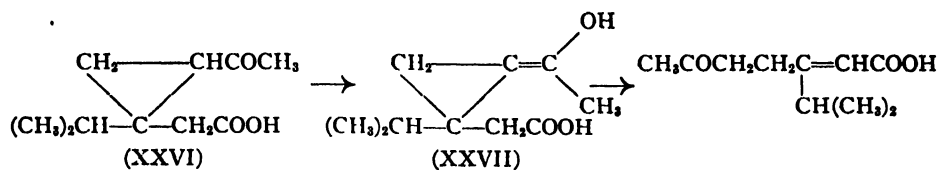
Dimethylallene adds hydrogen bromide to form a saturated dibromide  $((\text{CH}_3)_2\text{CBr} \cdot \text{CH}_2\text{CH}_2\text{Br})$  (12); whatever the mechanism the bromine atoms go to the ends of the system. When triphenyl allene is treated with hydrogen bromide it isomerizes to triphenylindene (15, 25); this may be represented as addition one way and subsequent elimination of a hydrogen from one of the benzene nuclei:



Similarly, methyl triphenylallene gives methyltriphenylindene (27).

After this mechanism was devised the literature was searched for all substances containing a cyclopropane ring with a hydroxyl group in the required position, and about a dozen compounds were found among the terpenes. In

every case the products actually formed by the action of sulphuric acid agreed with the predictions required by the above. Wallach (26) has suggested that an intermediate enol (XXVII) was formed to account for the unexpected ease of ring opening with the cyclic keto acid (XXVI). However, as this



substance is a cyclopropane ketone, it belongs to the class of substances the reactions of which are considered in the first paragraph, and this assumption is unnecessary.

## Experimental

### A. Preparation of the Cyclopropane Derivatives

Most of these were made using directions in the literature, but in some instances a variation of the procedure resulted in a better yield.

*Benzoylcyclopropane (III).*  $\gamma$ -Chlorobutyrophenone was prepared in a 50% yield following Conant's directions (10). Bruylants (6) predicted that the residual solutions should contain most of the other 50% as benzoylcyclopropane, so these were investigated as follows: the solution containing the ketimine was extracted with ether and the extracts from several runs allowed to accumulate until three litres had been obtained. The solvent was then distilled and the residue distilled with steam until diphenyl began to solidify in the condenser; the remainder of the diphenyl was collected separately but not quantitatively—benzoylcyclopropane is not volatile with steam. The oily layer in the first distillate was separated and carefully fractionated with the following results: 1.5 cc. of colorless liquid, b.p. 46° C., probably propyl chloride; 10 gm. of a colorless liquid, b.p. 78° C.,  $D_4^{22}$ , 0.8505, 0.8508 (*n*-butyl chloride has b.p. 77.9° C.,  $D_4^0$  C., 0.907, and  $D_4^{14}$ , 0.897 (21)); 20 gm. bromobenzene; 3 gm. diphenyl. The residual oil solidified and gave a small amount of a pale yellow solid, m.p. 177° C., which was not investigated. There was no indication of benzoylcyclopropane.

The cyclic ketone was prepared in a 93% yield by substituting potassium cyanide for the hydroxide; 10 gm. of  $\gamma$ -chlorobutyrophenone was added to 4 gm. of potassium cyanide in 100 cc. of absolute methyl alcohol and, since hydrogen cyanide was given off, was left in the hood for 10 days. The potassium chloride was filtered and the solvent allowed to evaporate, leaving the ketone which was then distilled *in vacuo*; 7 gm., b.p. 110–5° C. at 7 mm.; 248° C. at 760 mm. Absolute ethyl alcohol could be used, but the presence of water diminished the yield considerably. Cuprous cyanide acted in a similar manner, but the yield was only 60%; the residues did not give any  $\gamma$ -benzoylbutyric acid on hydrolysis, indicating no substitution of CN for Cl. Again, hydrogen cyanide was given off.



The oxime and semicarbazone were prepared and their melting points agreed with those already recorded. A 2,4-dinitrophenylhydrazone was made in the usual manner; it separated from benzene in fine red needles, m.p. 151°C. Analysis:—Calcd. for  $C_{16}H_{14}O_4N_4$ : N, 17.2%. Found: N, 16.9, 17.0%.

Methyl 1,3-diphenyl-2-benzoylcyclopropane-1-carboxylate (IX) was prepared in connection with another investigation and will be described in a later paper. Ethyl 1-cyanocyclopropane-1-carboxylate (XII) was secured in a yield of 76% following the procedure of Jones and Scott (13), but using equivalent quantities of halide and ester; ethylene dichloride and chlorobromide could not be substituted for the dibromide without greatly diminishing the yield.

*Diphenylcyclopropyl carbinol (XIV)*. Lipp (20) omitted the yield of this substance in his paper; the present authors did not succeed in obtaining more than 5% of the theoretical amount.

### B. Treatment with Sulphuric Acid

(a) *Benzoylcyclopropane (III)*. Sulphuric acid (2 cc.) was added to a solution of 5 gm. of the cyclic ketone in 40 cc. of glacial acetic acid and the mixture boiled for 5 min. The hot, brown solution was poured into water, extracted with ether and the extract washed with dilute sodium carbonate; 4 gm. of  $\gamma$ -acetoxybutyrophenone was obtained which after distillation had a boiling point of 195–200°C. at 40 mm. Analysis:—Calcd. for  $C_{13}H_{14}O_3$ : C, 69.9; H, 6.8;  $CH_3COO$ , 28.6%. Found: C, 69.4, 69.4; H, 6.3, 6.2;  $CH_3COO$ , 29.0%.

A mixture of 2.5 gm. of the acetate, 3.5 gm. of phenylhydrazine, 10 cc. of water and 30 cc. of alcohol was allowed to stand overnight. The 1,3-diphenyldihydropyridazine crystallized, and after purification had a melting point of 141°C.; a mixed melting point with a specimen prepared as directed by Conant (10) was not depressed.

The 2,4-dinitrophenylhydrazone, prepared in the usual manner, crystallized in thick red rods from benzene, m.p. 165°C. Analysis:—Calcd. for  $C_{18}H_{18}O_6N_4$ : N, 14.5%. Found: N, 14.8%.

(b) *Methyl 3-phenyl-2-benzoylcyclopropane 1, 1-dicarboxylate (VIII)*. A solution of 10 cc. of concentrated sulphuric acid, 5 gm. of the ester, and 25 cc. of acetic acid was refluxed for two hours, poured into water, and the mixture extracted with ether. The ether extract was washed first with dilute sodium carbonate solution, then dilute sodium hydroxide. On acidification of the carbonate solution a small amount of an acid, m.p. 118°C., was obtained; this substance was very unstable and could not be recrystallized because of the ease with which it decomposed into benzaldehyde and a solid acid, perhaps because of a trace of base present. A sample was refluxed with alkali for an hour and then distilled with steam; the benzaldehyde in the distillate was recognized by formation of the phenylhydrazone. The residue in the flask was acidified, and after a short time, an acid, m.p. 164–165°C., separated; phenacyl malonic acid (XVI) has a recorded melting point of 178°C. with

decomposition. Accordingly a specimen was prepared as directed in the literature (5), and carefully purified; it melted at 164–165° C., and a mixed melting point was also 164–165° C. On titration with standard alkali neutralization equivalents of 107.5 and 109 were obtained; the calculated value for  $C_{11}H_{10}O_6$  is 111.

A sample was heated at 180° C. as long as gas was given off, the residual oil dissolved in sodium carbonate, filtered and acidified.  $\beta$ -Benzoylpropionic acid was deposited and identified by comparison with a sample at hand; by dissolving 2 gm. of the cyclic ester in 20 cc. of concentrated sulphuric acid, pouring upon ice, extracting the oil with ether and hydrolyzing as above, the malonic acid was obtained in one step. A run of this size was carried out quantitatively and 1.2 gm. of acid (86% of the theoretical) found on titration with 0.5 *N* sodium hydroxide.

(c) *Methyl 1,3-diphenyl-2-benzoylcyclopropane-1-carboxylate (IX)*. This ester was recovered unchanged from solution in sulphuric acid, either alone or in acetic acid.

(d) *1,2-Dibenzoyl-3-phenylcyclopropane (V)*. Two stereoisomeric forms were used and both acted in the same manner. The conditions under which they were treated with the acid were varied considerably, but it was impossible to repeat any given procedure; often the cyclic compound was recovered unchanged. The details that gave the best results follow:—one cc. of sulphuric acid was added to 0.2 gm. of the cyclic compound in 10 cc. of acetic acid (for the isomer melting at 116° C.) and the mixture boiled for 5 min. It was then poured into water, extracted with ether and washed with sodium carbonate solution; a light yellowish amorphous solid remained after the ether was evaporated; it was purified by dissolving in chloroform and precipitated by pouring into methyl alcohol; m.p. 205° C. Analysis:—Found: C, 88.1; H, 5.3%; mol. wt., 586. The isomeric ketone (151°) required 25 cc. of acetic acid to dissolve it but the result was the same.

(e) *1-Phenyl-1-nitro-2-benzoylcyclopropane (XI)*. The isomer (m.p. 131° C.) was not affected when 1 gm. in 35 cc. of acetic acid containing 5 cc. of sulphuric acid was left overnight, or boiled for 1.5 hr. The mixture (m.p. 80° C.) was wholly converted into the form, m.p. 131° C. More concentrated solutions gave off oxides of nitrogen and left a brown oil from which nothing could be isolated; the oil did not give a 2,4-dinitrophenylhydrazone.

(f) *1-Nitro-2-(p-chlorobenzoyl)-3-phenylcyclopropane (X)*. This substance reacted slowly unless the solutions were moderately concentrated, but the furane was obtained in a yield of 95% by the following procedure: 5 cc. of concentrated sulphuric acid was added to a solution of 5 gm. of the cyclopropane in 75 cc. of acetic acid. Oxides of nitrogen were evolved on warming. After boiling for 10 min. the solution was poured into 400 cc. of water and extracted with ether; on removal of the solvent the furane was left (3.8 gm.). It was recrystallized from methyl alcohol and shown to be 2-phenyl-5-(p-chlorophenyl) furane (XVII) by a mixed melting point with a specimen prepared by the method in the literature (19); m.p. 123° C.

Diels and Alder have shown that simple furanes form addition products with maleic anhydride like dienes; this reaction was tried with the above furane but no matter what the conditions, the components were recovered unchanged. Since neither 2,5-diphenyl furane\* nor 2,3,5-triphenyl-4-bromofurane formed addition products with maleic anhydride, it would seem that in the furane series the reaction is blocked by two phenyl groups.

(g) *Ethyl 1-cyanocyclopropane-1-carboxylate (XII)*. The ester (5 gm.) was dissolved by stirring in 50 cc. of concentrated sulphuric acid and, after standing overnight, poured into 200 cc. of water. The oil was extracted with ether and thoroughly washed with sodium carbonate solution; on evaporation 2 gm. of the amide (XIX) was deposited, which after crystallizing from methyl alcohol formed thick white rods, m.p. 126° C. Analysis:—Calcd. for  $C_7H_{11}O_2N$ : N, 8.9%. Found: 8.8%.

The acid-amide (XX) was formed by refluxing for 20 min. 1 gm. of the ester-amide in 5 cc. of 30% aqueous solution of sodium hydroxide and enough alcohol to make a clear solution, pouring into water, acidifying and extracting five times with ether. The solid remaining after removal of the solvent was recrystallized from water, forming rectangular plates, m.p. 190° C. with decomposition. It dissolved instantly in alkaline solutions. Analysis:—Calcd. for  $C_6H_7O_2N$ : N, 10.8%. Found: N, 10.4%. A trace of another solid was found on ether extraction of the greatly concentrated aqueous solution, and recrystallized from benzene; m.p. 127° C. Analysis:—Found: C, 45.5; H, 5.1; N, 5.5%.†

The acid-amide was hydrolyzed to the known cyclopropane-1,1-dicarboxylic acid (XXI) by refluxing for 20 min. a solution of 2 gm. in 30% aqueous potash. It was isolated by ether extraction after acidification and the usual treatment. It melted at 136° C. and showed no depression when mixed with the acid as obtained by the established method (8).

As a reference compound the di-(*p*-bromophenacyl) ester was prepared. A solution of 0.4 gm. of the acid in 5 cc. of water was neutralized with sodium carbonate and then just barely acidified to litmus with hydrochloric acid; 10 cc. of alcohol was added and water to keep the salt from separating—then 0.8 gm. of *p*-bromophenacyl bromide was introduced and the mixture refluxed for an hour. The ester separated on cooling, and after recrystallization from alcohol formed long needles, m.p. 146° C. Analysis:—Calcd. for  $C_{21}H_{16}O_6Br_2$ : Br, 30.5%. Found: Br, 29.9%.

(h) *1,3-Diphenyl-2-benzoyl-1-cyanocyclopropane (XIII)*. Three stereoisomeric forms of this were available from a previous investigation; they all gave the same amide (XXIII). Five grams of the cyclic nitrile (m.p. 166° C.) was dissolved in 50 cc. of concentrated sulphuric acid by mechanical stirring, and left overnight. After pouring into 450 cc. of water the white precipitate

\*The experiments with this substance were performed by Mr. C. S. Maxwell.

†Owing to the small amounts found its structure was not determined; the analysis corresponds to  $C_{10}H_{13}O_2N$ , or one molecule of amide-acid plus one molecule of di-acid.

was filtered and recrystallized from acetic acid, in long rods, m.p. 179° C.; 2.5 gm. was obtained. Analysis: \*—Calcd. for  $C_{23}H_{19}O_2N$ ; C, 80.9; H, 5.6%. Found: C, 81.0; H, 5.3%.

A small amount (0.2 gm.) of an isomeric amide (m.p. 198° C.) was insoluble in the acetic acid, but was purified by the use of methyl alcohol, and found to be identical with the amide prepared in another investigation, by the action of ammonia on the corresponding cyclic ester.†

*Isomerization.* A mixture of 0.2 gm. of the amide (m.p. 179° C.), one drop of sulphuric acid and 20 cc. of acetic anhydride was kept at 100° C. for two hours, decomposed by iced carbonate and worked up in the usual way. The product was shown to be the isomeric form (m.p. 198° C.) by a comparison of solubilities, melting point and mixed melting point.

The reverse change was brought about by saturating a methyl alcoholic solution of 1 gm. of the amide (m.p. 198° C.) with hydrogen chloride and after several days removing the solvent and crystallizing the residue from benzene; by a comparison of properties and mixed melting point it was found to be the isomer melting at 179° C.

*Dehydration.* The amide (179° C.) was recovered unchanged after refluxing with thionyl or acetyl chloride. A mixture of 0.5 gm. each of the amide and phosphorus pentachloride in 25 cc. of dry xylene was refluxed for 40 min. The phosphorus compounds were washed out with water and the nitrile (m.p. 166°) crystallized on partial removal of the solvent; it melted at 164–165° and a mixed melting point was 165–166° C. Neither amide was esterified under any conditions.

(i) *Diphenylcyclopropyl carbinol (XIV)*. This substance dissolved in sulphuric acid with the production of an orange-red color, slowly changing to green. It could not be recovered, even on immediate addition of the solution to ice. The oil produced was unsaturated, decolorizing bromine and permanganate, but gave no ester in the Schotten-Baumann reaction. The amount available was too small to make further study practical.

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\*For this analysis the writers are indebted to Mr. H. B. Rosener.

†The results of this work will be published later.

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## DIHYDRO-*p*-TOLUALDEHYDE<sup>1</sup>

By C. F. H. ALLEN<sup>2</sup>, W. L. BALL<sup>3</sup> AND D. M. YOUNG<sup>4</sup>

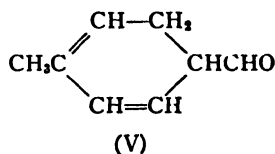
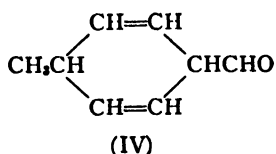
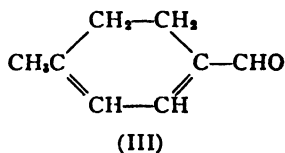
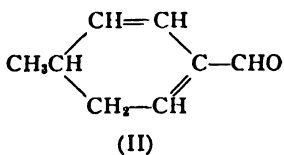
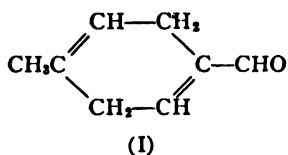
### Abstract

In the condensation of acetaldehyde to crotonaldehyde, dihydro-*p*-tolualdehyde is obtained as a by-product. The determination of its structure, its properties and some of its reactions are described.

In the manufacture of crotonaldehyde there is always a certain amount of oily residue left in the still. When the residue is submitted to distillation, a yellow oil is obtained. The oil decolorizes bromine and permanganate, and gives an addition product with sodium bisulfite—this would indicate an unsaturated aldehyde or methyl ketone.

Samples of several lots of this oil were placed at the writers' disposal\* and the problem of determining the structure of one or more of the substances present was attacked.

When submitted to a slow fractionation *in vacuo*, two main fractions were collected, A (b.p. 68°C. at 10 mm.) and B (b.p. 77°C. at 10 mm.). Both of these decolorize bromine instantly but in a short time evolve hydrogen bromide, reduce permanganate rapidly, form addition products with sodium bisulfite, and reduce Fehling's solution; with semicarbazide, 2,4-dinitrophenylhydrazine, hydrazine, and cyanoacetic acid they both give the same derivatives; therefore they would seem to be unsaturated aldehydes. Upon oxidation with nitric acid, both give *p*-toluic acid; with permanganate the oxidation proceeds to terephthalic acid. In view of these properties the substances are derivatives of dihydrobenzene, having a methyl group in the para position to the aldehyde group. Five structures are thus possible:



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Contribution from the Chemical Laboratory of McGill University, Montreal, Canada.

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\*Generous specimens of these oils were very kindly furnished by Shawinigan Chemicals Limited, for which the authors express their appreciation.

Since structures (II, III, and V) contain a conjugated system, a study of the addition products with maleic anhydride or  $\alpha$ -naphthoquinone might be expected to give a clue as to the nature of the substance, but neither of the fractions reacts, the anhydride or quinone recrystallizing unchanged. The aldehydes do not add to 1,4-diphenylbutadiene. These facts cannot be used to exclude the three structures, for it is only when the Diels-Alder reaction is positive that it is of value in the determination of structure (5). Ozone slowly oxidizes both but the oxidation product is *p*-toluic acid. Autoxidation also gives the same acid.

The distinction between A and B is based entirely on physical properties since in every instance they both give the same derivatives—the identity of the latter was determined by melting points, mixed melting points, solubilities and crystallographic properties.\* The reactions forming these derivatives are very rapid and give almost the same yield, though slightly smaller from A. Both A and B are completely soluble in a saturated aqueous solution of sodium bisulfite, but when the aldehyde is regenerated, B alone is found, and its physical properties are slightly different. B is also regenerated from the semicarbazone. This would indicate that A contains some other substance, but so far none has been isolated.

The physical properties are shown in Table I, along with *p*-tolualdehyde and hexadienal for comparison.

TABLE I

Substance	Boiling point, °C.		Density <sup>20°</sup> <sub>4</sub>	$n_D^{20}$	Molecular refractivity
	At 10 mm.	At 760 mm.			
A	68	195	1.0035	1.5182	36.854
B	77		1.0150	1.5279	37.006
Regenerated from NaHSO <sub>3</sub> compound	77	202	1.0176	1.5408	37.661
<i>p</i> -Tolualdehyde	95	203	1.0297	1.5465	37.546
Hexadienal*	64–6/11	173–4/754	0.9087 <sup>21</sup> <sub>4</sub>	1.5372 <sup>21</sup> <sub>D</sub>	

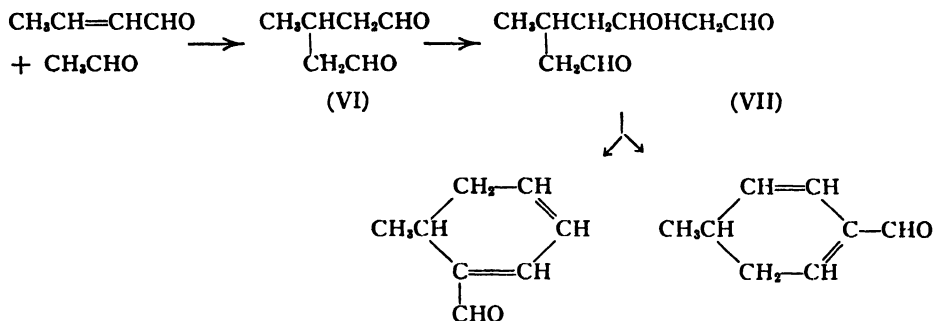
\*Octatrienal is a solid, m.p. 55°C., that polymerizes in a few hours (6).

The aldehyde was condensed with acetone; the product formed had an odor like benzalacetone rather than ionone. Both the pure aldehyde and unsaturated ketone may prove of use to the perfumer.

In a series of three papers (2,3,4) Bernhauer has described certain oils obtained in the preparation of crotonaldehyde under conditions not stated. One was shown to be dihydro-*o*-tolualdehyde, and another, from the meagre evidence given, is doubtless the corresponding para compound here described. In the oils used by the writers there has been no trace of the ortho compound, although a careful examination was made for it. The location of the double bonds in the ortho aldehyde was not determined.

\*These were determined by Professor R. P. D. Graham of the Department of Mineralogy, to whom the authors acknowledge their indebtedness.

A reasonable mechanism that accounts well for the formation of both isomers and that is based on the assumption that acetaldehyde is always present, whether initially or produced by hydrolysis of crotonaldehyde\*, is proposed by the present authors as follows: a molecule of acetaldehyde adds to the conjugated system of crotonaldehyde, and the dialdehyde (VI) then adds a second molecule of acetaldehyde, to form (VII). This can condense with itself in two ways, one of which leads to the dihydro-*p*-tolualdehyde and the other to the ortho isomer.



The location of the double bonds is uncertain. One pair is probably conjugated with the side chain carbonyl group since the molecular refraction shows an exaltation (calculated value, if no exaltation = 36.224); because of the ease of removal of two hydrogen atoms, formula (I) may be favored.

### Experimental

The aldehyde-containing specimens of oil received were all slowly fractionally distilled in the same apparatus at 10 mm. pressure; a partial condenser containing technical *n*-butyraldehyde was used, to prevent high boiling material from distilling over. It was found that the rate of distillation had a marked effect on the boiling point as registered by the thermometer, superheating being very pronounced. In the apparatus used the containing flask was heated by a hot-water bath, at such a rate that four drops of the distillate every five seconds was collected from the end of the condenser. The apparatus was filled with carbon dioxide. A very small low boiling fraction and the residue were kept separate for future study. Fraction A: b.p. 68° C. at 10 mm.;  $D_4^{20}$  1.0035;  $n_D^{20}$  1.5182. Fraction B: b.p. 77° C. at 10 mm.;  $D_4^{20}$  1.0150;  $n_D^{20}$  1.5279. Both were a very pale yellow but darkened on standing, and had a rather pleasant but very penetrating and slightly soapy odor; this was not present in the aldehyde purified through the bisulfite compound.

Both fractions were shaken with a saturated solution of sodium bisulfite, and the precipitated addition product filtered and washed thoroughly with ether. It was then decomposed by mixing with slightly more than the calculated amount of potassium carbonate, and water, and steam distilling.

\*Kuhn has shown that PURE crotonaldehyde is not convertible into hexadienal or octatrienal by catalysts (6).



The aldehyde was extracted with ether, the extract dried with potassium carbonate and, after removal of the solvent, distilled *in vacuo*. The pure dihydro-*p*-tolualdehyde is a very highly refractive, colorless liquid, possessing a sweet, pleasant odor like *p*-tolualdehyde (or English hawthorn blossoms); b.p. 77° at 10 mm., 202°C. at 760 mm.;  $D_{20}^{20}$  1.0176;  $n_D^{20}$  1.5408;  $M_D$  37.661 (calcd.  $M_D$  for (I) = 36.954). The same constants were obtained with a sample regenerated from the semicarbazone. Analysis:—Calcd. for  $C_8H_{10}O$ : C, 78.6; H, 8.2%. Found: C, 78.6; H, 8.0%.

Dihydro-*p*-tolualdehyde is easily miscible with the usual organic solvents but does not dissolve appreciably in water, dilute acids, or alkalis. It is charred by concentrated sulphuric acid, and becomes dark brown in the presence of alkalis. It is very easily distilled with steam, the distillate being about one-seventh aldehyde.

It does not give a color with Schiff's reagent, but readily reduces Fehling's solution, especially if a little alcohol is added. It dissolves completely in sodium bisulfite solution; if the solution is saturated, the addition product separates in lustrous leaflets.

This aldehyde very easily undergoes autoxidation, but the process is prevented by addition of a little hydroquinone. It rapidly and instantly decolorizes bromine, but, after a short time, if at all concentrated, hydrogen bromide is given off; under the same conditions *p*-tolualdehyde is unchanged. It consumes far less than the calculated amount of bromine—a similar observation was made by Kuhn with the polyene aldehydes (6)—the amount changing with slight variations in operating; *e.g.*, on the assumption that one mole of bromine per mole of aldehyde is 100%, the following values were obtained (bromide-bromate procedure, or direct titration) 52.95, 34.47, 50.59, 41.43, 33.53, whereas pure *p*-tolualdehyde gave only 0.43.

On heating in an inert atmosphere for several hours it was unchanged; when maleic anhydride or  $\alpha$ -naphthoquinone was added and the mixture heated for several hours, there was no reaction, the anhydride and quinone crystallizing on cooling.

The 2,4-dinitrophenylhydrazone was prepared in the usual manner (1). It is very soluble in hot xylene or *p*-cymene, sparingly soluble in hot *n*-butyl alcohol, and insoluble in the other usual solvents; xylene is the best medium for purification. It forms brick-red plates with square ends, m.p. 239°C. Analysis:—Calcd. for  $C_{14}H_{11}O_4N_4 \cdot H_2O$ : N, 17.5%. Found: N, 17.7, 17.9, 17.6%. *p*-Tolualdehyde 2,4-dinitrophenylhydrazone was prepared for comparison; it crystallizes in pointed rods from xylene or cymene, m.p. 206°C.

The semicarbazone, when prepared in the usual manner, formed long transparent needles with square ends. It is moderately soluble in ethyl alcohol but dissolves freely in ethyl acetate. In a capillary tube, it shrinks slightly at about 215°C., melting to a clear liquid at 219°C.; when mixed with the semicarbazone of *p*-tolualdehyde it shrunk at 214° and melted at 217–219°C. Although there is no marked depression of the mixed melting point, the crystals are entirely different in shape, size and other properties.

The *p*-nitrophenylhydrazone was an amorphous substance that could not be crystallized.

*The cyano acid*;  $C_7H_9CH=C(CN)COOH$ . A solution of 2 gm. of sodium hydroxide, 30 cc. of water, and 4 gm. of cyanoacetic acid was made slightly alkaline to litmus by adding a little aqueous solution of sodium hydroxide, placed under a stirrer and 5 gm. of the aldehyde introduced. After warming and stirring for three minutes the reaction was completed, and the condensation product was precipitated by addition of concentrated hydrochloric acid. The solid was filtered and recrystallized from dilute alcohol, forming pale yellow needles and rods, m.p. 212-213°C. Analysis:—Calcd. for  $C_{11}H_{11}O_2N$ : N, 7.4%. Found: N, 7.3%.

*The aldazine*;  $C_7H_9CH=N-N=CHC_7H_9$ . A mixture of 5 gm. of hydrazine sulfate, 10 gm. of potassium acetate, and 50 cc. of hot water was cooled slightly, and 50 cc. of alcohol added. The precipitated salt was filtered and 10 gm. of aldehyde was added; the azine separated in a few minutes and was filtered when cold. It was recrystallized from *n*-propyl alcohol, in which it is very soluble hot but practically insoluble cold, forming pale yellow, wedge-shaped prisms, m.p. 157°C. It is insoluble in the cold alcohols, and only sparingly soluble in hot ethyl alcohol, but dissolves readily in ether or hot propyl alcohol. It slowly goes into solution in concentrated sulphuric acid with formation of a bright yellow color.

A cinchoninic acid (from the aldehyde,  $\alpha$ -naphthylamine, and pyruvic acid) was obtained in such a small yield it was not extensively purified.

*Oxidation.* (a) *Nitric Acid*. In a 250-cc. three-necked flask fitted with a stirrer and reflux condenser were placed 5 gm. of the aldehyde and the dilute nitric acid made up from 25 cc. of acid (sp. gr. 1.42) and 75 cc. of water, and the whole heated an hour on the steam bath; the *p*-toluic acid separated on cooling. It was filtered, and weighed 3.5 gm. (64%), m.p. 176-177; mixed m.p. 176-177°C.

(b) *Potassium permanganate*. In the same apparatus 40 cc. of acetone, 30 cc. of water and 10 gm. of aldehyde were heated to boiling on the steam bath and 50 gm. of permanganate added in 10-gm. portions as the course of the reaction permitted. After an hour the oxidation was considered completed, though traces of toluic acid were found later on. The oxides of manganese were filtered and extracted with boiling water; on acidification of the filtrate, terephthalic acid was precipitated. It was dissolved in aqueous sodium bicarbonate and reprecipitated by hydrochloric acid; yield, 8 gm. or 66%. For identification a sample was converted into the dimethyl ester by the usual procedure; m.p. 135-137°; mixed m.p. 136-137°C.

(c) *Ozonization*. This was done only qualitatively; after four hours unchanged aldehyde was still present, as shown by removal with sodium bisulfite and formation of the dinitrophenylhydrazone. The alkaline extract of the ethereal solution gave *p*-toluic acid on acidification.

(d) *Autoxidation*. A sample left exposed on a watch glass was nearly solid after three days; the solid was *p*-toluic acid. A similar sample containing hydroquinone was apparently unchanged in the same time.

*Condensation with acetone*. A mixture of 50 gm. of the aldehyde, 100 cc. of acetone, 500 cc. of water and 10 gm. of barium hydroxide was shaken mechanically for 14 hr., acidified, and steam distilled. The residual oil was taken up in ether and benzene, the extract dried, and after removal of the solvent the unsaturated ketone was distilled in an atmosphere of carbon dioxide; b.p., 177-185°C. at 25 mm. The yield was low (15.3 gm., 23%), there being much high boiling product and tar. The odor resembled that of benzalacetone. The semicarbazone, prepared as usual, crystallized in white rods from ethyl acetate; in a capillary tube it shrunk at 196°, and melted at 206-210°C. with decomposition. Analysis:—Calcd. for  $C_{12}H_{17}ON_3$ : N, 19.2%. Found: N, 18.5%. The unsaturated ketone gives a bright red color with concentrated sulphuric acid.

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## PYROLYSIS OF THE LOWER PARAFFINS

### II. THE PRODUCTION OF OLEFINES IN BAFFLED QUARTZ TUBES<sup>1</sup>

BY ADRIEN CAMBRON<sup>2</sup> AND COLIN H. BAYLEY<sup>3</sup>

#### Abstract

The pyrolysis of the lower paraffins in externally heated quartz tubes has been studied under conditions of streamline and turbulent flow. It has been found possible to produce a high degree of turbulence in the flow of paraffin gases through tubes heated to a high temperature. It has been shown that when the pyrolysis is carried out under conditions of turbulent flow the yields of olefines obtained at a given temperature are greatly increased over those obtained in an open tube. It is further shown that under conditions of turbulent flow higher rates of conversion of the lower paraffins to olefines are possible since the temperatures at which side reactions begin to be noticeable are considerably higher under the above conditions than when the gas flow is streamline.

A considerable amount of work has been done on the effect of such factors as temperature, time of contact and ratio of reaction space to surface on the conversion of the lower paraffins to olefines. The work done on this subject as well as on the use of catalysts has been comprehensively reviewed by Lomax *et al.* (10), their review containing a chronological index of the literature from 1809 to 1915. A later and more elaborate review by Egloff, Schaad and Lowry (5) covers the literature up to 1930. Experiments carried out in these laboratories on the conversion of the lower paraffin hydrocarbons to olefines, by passing the gases over a glowing tungsten spiral or carbon rod, were reported in a previous communication (2).

It is generally accepted that in problems of heat transfer from a solid surface to a gas, one of the controlling factors is the resistance to heat flow of the stationary film of gas which covers the surface. The amount of heat absorbed by the gas in the form of radiation is small, even at the temperatures employed in pyrolysis experiments, so that the greater part of the heat transfer takes place by conduction through the stationary gas film (8,9,14).

The rate of heat transfer between a heated surface and a gas is consequently assumed to depend to a great extent on the thickness of the surface film, which in turn varies to some extent with the viscosity of the gas and with the temperature, but to a greater degree with the character of the gas flow near the surface, for a rapid movement of the gas in this region such as is caused by turbulence, for instance, tends to break down the film.

It is well known that the reactions involved in the conversion of paraffins to olefines are endothermic. Thus the dehydrogenation of the lower paraffins to the corresponding olefines requires about 32,000 calories per gram-mole, whilst the splitting up of the paraffins containing more than two carbon atoms into an olefine and a lower paraffin requires about 16,500 calories per

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gram-mole. It follows, therefore, that if a gaseous paraffin (or the vapor of a low-boiling paraffin) be passed through a reaction tube the walls of which are heated to a temperature sufficiently high to cause splitting up of the paraffin into olefines, the amount of reaction taking place after the gas has reached reaction temperature will to some extent be proportional, for a given time of contact, to the rate of heat transfer between the tube wall and the gas. It is known that under certain conditions, for example, at linear velocities greater than a critical value for a tube of a given diameter, the flow of gas in the tube becomes turbulent. It was therefore thought of interest to estimate the critical velocities at which the flow of the lower paraffins changes from streamline to turbulent in a 2.6-cm. tube, such as used in the present experiments.

Using the value of 2,500 for Reynold's number as determined by Stanton and Pannel (12) the critical velocity can be calculated from the following equation:

$$V_c = \frac{2500 \mu}{d\rho}$$

where  $V_c$  is the critical velocity in cm./sec.;  $\mu$ , the viscosity in C.G.S. units;  $d$ , the diameter of the tube in cm.; and  $\rho$ , the density of the gas in gm./cc.

Substituting the appropriate values for the lower paraffins, starting with ethane, the values shown in Table I are obtained for a 2.6-cm. tube at room temperatures.

TABLE I  
CRITICAL RATES IN 2.6-cm. TUBE AT ROOM TEMPERATURE

Gas	$\mu$	$\rho$	Linear velocity, cm./sec.	Critical rate in l/hr. in 2.6-cm. tube
Ethane	0.0000929	0.00134	66.5	1270
Propane	.0000806	.00200	39.0	745
Butane	.0000739	.00260	27.0	515

It will be observed that, in the above equation, owing to the concurrent increase in density and decrease in viscosity as the length of the paraffin chain increases, there is a rapid decrease in critical velocity with increase of molecular weight so that if pentane vapor, at 40°C. for instance, were passed through a 2.6-cm. tube at 400 litres per hour the gas flow would be expected to be turbulent.

The rate of thermal decomposition of the lower paraffins becomes rapid at temperatures of 800–1000°C. and it is consequently in this temperature range that the estimation of the critical velocity was believed to be of interest. Knowing the critical velocity at room temperature it is possible to calculate approximately the critical velocity at say 900°C., if one disregards such disturbing effects as convection in that part of the tube where the temperature of the gas is different from the temperature of the tube wall, and assuming that Reynold's number does not vary appreciably with temperature.

It will be observed that, according to the equation:

$$V_c = \frac{2500 \mu}{d\rho},$$

the expansion of the gas due to increase in temperature will cause an increase in the linear velocity, but also a corresponding decrease in the density, so that, assuming the viscosity to be constant, the critical mass velocity would remain constant. The viscosity coefficient,  $\mu$ , however, increases rapidly with the temperature, causing a corresponding increase in the critical velocity, so that we have the relation:

$$\frac{(V_c)_T}{(V_c)_o} = \frac{\mu_T}{\mu_o},$$

where  $(V_c)_T$ , is the critical velocity at temperature  $T$ ;  $\mu_T$ , the coefficient of viscosity at temperature  $T$ ;  $(V_c)_o$ , the critical velocity at 20°C.; and  $\mu_o$ , the coefficient of viscosity at 20°C.

Although Sutherland's formula for the variation of viscosity with temperature is not accurate at higher temperatures, its use in this case probably gives a sufficiently close approximation of the value of the critical velocity, and it should give a fairly good idea of the variation of the critical velocity with the molecular weight of the paraffin.

According to Sutherland's formula:

$$\frac{\mu_T}{\mu_o} = \left( \frac{T}{273} \right)^{\frac{3}{2}} \frac{C+273}{C+T}$$

where  $T$  is the temperature of the gas, and  $C$ , Sutherland's constant.

Table II, shows the values of  $C$  taken from Landolt, the value of the viscosity coefficient for each gas at 900°C. as calculated by the above formula, the value of  $V_c$  reduced to 20°C. and the critical rate of each gas in litres per hour in a 2.6-cm. tube.

TABLE II  
CRITICAL RATES IN 2.6-cm. TUBE AT 900°C.

Gas	$C$	$\mu$ , at 900°C.	$V_c$ , cm./sec.	Rate in litres/hr. in a 2.6-cm. tube
Ethane	287.3	0.000325	232	4440
Propane	241.3	0.000290	140	2680
Butane	377.4	0.000273	100	1900

The increase in critical velocity was considerably higher than was expected and the wide difference between the critical rates and the rates ordinarily used in pyrolysis experiments indicates that the gas flow in unobstructed tubes at temperatures around 900°C. is undoubtedly streamline throughout the greater part of the tube.

### Effect of Baffles on the Gas Flow

Preliminary experiments having shown that a series of circular disks placed centrally in the tube were highly efficient in producing turbulence, a series of experiments was carried out to determine the relation between the diameter and the spacing of the disks or baffles and the degree of turbulence produced.

The degree of turbulence was taken as proportional to the loss of kinetic energy of the gas flowing through the baffled tube, this loss being determined by measuring the pressure drop over the length of the tube by means of static pressure tubes connected to manometers.

The following experiments were carried out at room temperature partly for convenience and partly by necessity, since, at the higher temperatures accurate measurements of pressure drop could not have been made.

The first series of experiments (Table III) was carried out with mica baffles 2.3 cm. in diameter in a 2.6-cm. tube of glazed translucent quartz, the baffles being supported on a 0.9-cm. diameter quartz rod which was placed at the axis of the 2.6-cm. tube, the baffled length being 42 cm. The second series of experiments (Table IV) was carried out in the same manner, except that the diameter of the baffles was 2.1 cm. instead of 2.3 cm. In these two tables the first vertical column gives the gas rate in litres per hour, propane being the gas used, and the first horizontal column gives the spacing between the baffles, in centimetres. The other figures give the pressure drop in centimetres of water between the two ends of the tube corresponding to a given rate of 1400 litres per hour; the pressure drop was too small to be measurable on the manometers used.

TABLE III

RELATION BETWEEN PRESSURE DROP, GAS RATE AND BAFFLE SPACING WITH 2.3-cm. BAFFLES

Rate, l/hr.	Baffle spacing, cm.									
	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.8
	Pressure drop, cm. of water									
428	0.33	0.31	0.35	0.35	0.35	0.34	0.34	0.31	0.28	0.23
507	.44	.42	.47	.44	.46	.45	.42	.41	.37	.32
625	.67	.65	.70	.63	.72	.68	.66	.68	.63	.52
730	.89	.85	.94	.89	.96	.92	.90	.96	.84	.70
798	1.11	1.06	1.17	1.05	1.19	1.13	1.13	1.14	1.04	.91
893	1.30	1.25	1.40	1.24	1.43	1.36	1.35	1.37	1.28	1.06
968	1.51	1.46	1.62	1.42	1.65	1.56	1.57	1.59	1.48	1.25
1070	1.82	1.76	1.98	1.75	1.96	1.90	1.94	1.95	1.83	1.53
1170	2.20	2.16	2.41	2.16	2.34	2.30	2.33	2.37	2.24	1.92
1325	2.71	2.75	2.98	2.80	2.98	2.84	2.88	2.94	2.84	2.49
1445	3.08	3.04	3.45	3.33	3.49	3.32	3.33	3.40	3.27	2.90

NOTE:—2.6-cm. tube.

TABLE IV

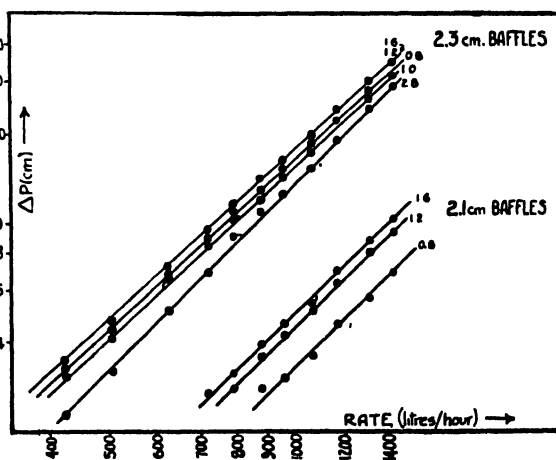
RELATION BETWEEN PRESSURE DROP, GAS RATE AND BAFFLE SPACING WITH 2.1-cm. BAFFLES

Rate, l/hr.	Baffle spacing, cm.			
	0.8	1.2	1.6	2.0
	Pressure drop, cm. of water			
428	0.06	0.08	0.08	0.06
507	.08	.12	.12	.10
625	.11	.16	.18	.17
730	.16	.24	.27	.23
798	.20	.28	.32	.29
893	.28	.36	.39	.38
968	.30	.42	.46	.43
1070	.36	.51	.53	.54
1170	.46	.63	.68	.65
1325	.56	.79	.87	.84
1445	.68	.93	1.02	1.00

NOTE:—2.6-cm. tube.

Some of the values obtained in the above experiments have been plotted (Fig. 1). In these curves the log of the pressure drop in centimetres ( $\log \Delta P$ ) has been plotted against the log of the gas rate.

The relation between pressure drop and gas rate and also the fact that the pressure drop is, within limits, independent of the number of baffles, show quite definitely that the baffles produce turbulence in the gas flow when propane is passed through the tube at rates above 400 litres per hour. With regard to the relation between pressure drop ( $\Delta P$ ) and rate of flow ( $V$ ), when the flow is streamline the pressure drop varies directly with the gas velocity; for a given gas and a given size and length of tube

Fig. 1. Relation between  $\log \Delta P$  and  $\log$  of gas rate for different baffle spacings and diameters.

$$\Delta P = KV.$$

When the flow is turbulent, however, the pressure drop varies with the square of the gas velocity

$$\Delta P = KV^2$$

$$\text{or } \log \Delta P = K + 2 \log V,$$



and this is actually the relation that has been found to exist between pressure drop and rate in a baffled tube. It will be observed (Fig. 1) that the plot of  $\log \Delta P$  against  $\log V$  gives straight lines and that the slope of the curves is very close to 2 ( $\log \Delta P$  is plotted on half the scale of  $\log V$ ) except when the baffle spacing is increased beyond 2.4 cm. The fact that the slope of the curve varies slightly from the theoretical value may be due to defects in the static pressure tubes used to determine the pressure at each end of the baffled tubes. The curves for the 1.4, 1.8, 2.0, 2.2 and 2.4-cm. baffle spacing for the 2.3-cm. baffles have not been plotted because these lie very close to the curves for 1.2-cm. spacing.

The fact that all the curves are parallel show that although the degree of turbulence varies with the spacing and the diameter of the baffles, the flow remains turbulent within the limits used. According to a recent paper by Colburn and King (3) the heat transfer from a heated tube to a gas passing through the tube under conditions of turbulent flow varies with somewhat less than the 4th power of the pressure drop, and in view of this relation between the heat transfer rate and the pressure drop it would be expected that, since the pressure drop does not vary appreciably when the baffle spacing is increased from 1.2 to 2.8-cm., the heat transfer rate and consequently the amount of decomposition which takes place when a paraffin is passed through a heated tube should be more or less constant within these limits.

This has actually been found to be the case as the results of experiments which have been carried out to test this point. These experiments show that varying the baffle spacing between 1.2 and 2.8 cm. had no appreciable effect on the rate of pyrolysis of propane, or on the course of the reaction at a given temperature and at a gas rate of 400 litres per hour.

Furthermore, in view of the relation between pressure drop and heat transfer rate, it will be observed that with the 1.6-cm. spacing, for example (2.3-cm. baffles), the pressure drop increases from 0.35 cm. at 428 litres per hour to 1.19 cm. at 798 litres per hour. According to Colburn and King heat transfer coefficients under these conditions would be expected to vary approximately with the 0.4 power of the pressure drop. Consequently the relation between the heat transfer rates at 798( $H_2$ ) and 428( $H_1$ ) litres per hour would be:—

$$\frac{H_2}{H_1} = \frac{1.19^{0.4}}{0.35^{0.4}} = 1.6$$

According to these figures, increasing the gas rate in the baffled tube should result in a very considerable increase in the heat transfer rate and consequently, in the degree of pyrolysis of the paraffins; the capacity and current efficiency of a given reaction tube should increase with the gas rate, provided, of course, other factors such as temperature of tube wall and contact time were kept constant.

## Thermal Dehydrogenation of Paraffins under Conditions of Streamline and Turbulent Flow

### Apparatus

The apparatus used in the present experiments is shown in Fig. 2. The gas pressure was reduced to about 20 lb. by means of a reducing valve and the gas flow regulated through a low pressure valve (*B*) whence the gas passed to the capillary flowmeter (*C*), the wetmeter (*D*), the drying tower (*E*) containing fused calcium chloride and to the furnace *F*. The quartz rod carrying the mica baffles (*G*) was supported usually at three points by triangles of heavy mica sheet mounted on the rod and fitting the tube snugly. The

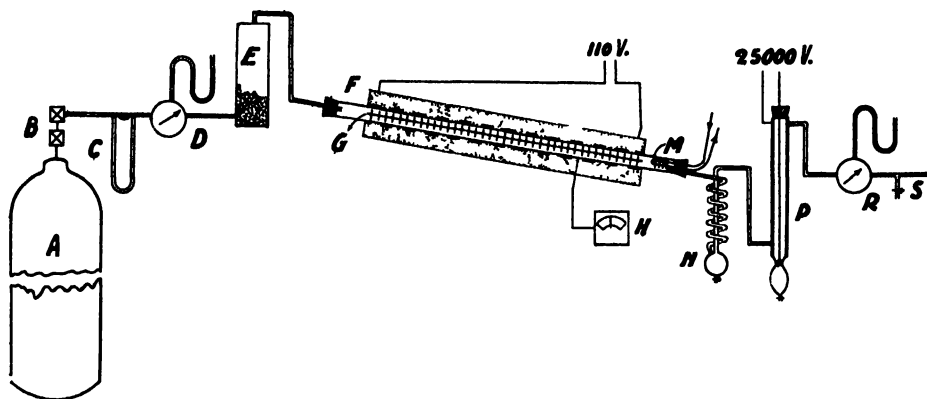


Fig. 2. Diagram of apparatus.

gas leaving the furnace was rapidly cooled by means of a loop of  $\frac{3}{16}$ -in. copper tubing to which a number of copper baffles (*M*) were soldered, and through which cold water was passed. The gas then passed through a spiral glass condenser (*N*) immersed in cold water, an electrostatic precipitator (*P*), and a wet meter (*R*). Samples of the exit gas were taken for analysis through the tube (*S*).

The gas was analyzed over mercury in an Orsat apparatus, the reagents being contained in Francis bubblers. Alkaline mercuric cyanide (13) was used for the determination of acetylene, whilst the butylenes and propylene were determined by absorption in sulphuric acid of 62.4 and 82.5% strength respectively, according to Hurd and Spence's modification (6) of Dobrjanski's method (4). A 4% bromine solution in 10% aqueous potassium bromide was used for the determination of ethylene, the back of the pipette being fitted with a pressure bulb by which the gas could be freed from bromine vapor by passage into potash before transferring to the burette. Hydrogen was determined by fractional combustion over copper oxide at 300°C. The carbon number of the paraffin residue was obtained by slow combustion in the apparatus described by Bayley (1).

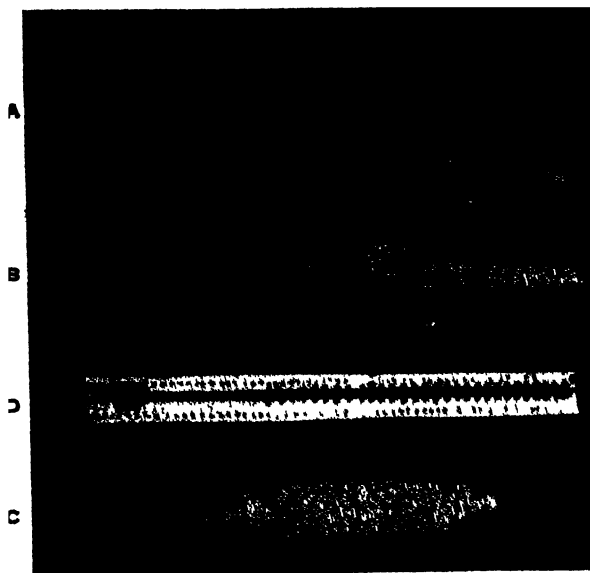


Fig. 3. Quartz reaction tubes used in these experiments, and a set of baffles for the 80-cm. furnace.

Fig. 3. shows three of the furnaces and a set of baffles used in the present experiments. (A) is the 80-cm. furnace with a tube of 2.5 cm. diameter, (B) is the 20-cm. furnace with a tube of 2.7 cm. diameter and (C) is the 18.5 by 2.0 cm. tube. (D) shows the baffles as used in each section of the 80 by 2.5 cm. furnace.

#### Gases Used

The ethane, propane and *n*-butane were supplied in cylinders by the Carbide and Carbon Chemicals Corporation. The isobutane used was the commercial grade supplied by the Viking Company of Charleston, West Virginia. The values of *n* in  $C_nH_{2n} + 2$  for each paraffin, determined by slow combustion analysis, are given in Table V.

TABLE V  
VALUES OF *n* FOR SAMPLES OF PARAFFINS USED

Gas	Ethane	Propane	<i>n</i> -Butane	Isobutane
<i>n</i>	1.93	2.95	3.98	4.07

#### (a) EFFECT OF BAFFLES ON WALL TEMPERATURES

The increase in the rate of heat transfer from tube wall to gas is well illustrated in the following experiments with propane. On passing the gas at the rate of 400 litres per hour through a furnace consisting of a 40 cm. by 2.5-cm. diameter quartz tube the results shown in Table VI were obtained with and without the use of baffles respectively.

TABLE VI  
COMPARISON OF PYROLYSIS OF PROPANE IN EMPTY AND IN BAFFLED TUBES AT THE SAME CURRENT INPUT

	Empty tube	Baffled tube		Empty tube	Baffled tube
Gas rate, l/hr.	400	400	Analysis of gaseous product, % by volume		
Current, watts	1113	1113	$C_3H_8$	1.5	0.3
Wall temp., °C.	996	918	$C_2H_4$	20.7	20.7
Expansion, %	55.7	58.9	$C_2H_6$	6.6	11.7
Gm. olefines per hr.	240.0	301.0	$H_2$	17.1	13.8
By-products	Liquids and tar	None			

The wall temperatures recorded in these experiments were the temperatures indicated by thermocouples placed against the outside surface of the reaction tube, and near the outlet end of the heated section where the wall temperature was highest.

The effect of the turbulence produced by the baffles on the rate of heat transfer between the tube wall and the gas is clearly shown by the difference in the tube wall temperatures in the empty and the baffled tube, the difference being obviously due to the fact that heat is being removed from the wall at a faster rate when the gas flow is turbulent.

It will also be observed that the amount of reaction is about the same in both experiments, although the wall temperature was 78°C. lower in the baffled tube. This shows that under conditions of substantially streamline flow, as the writers have shown to be the case in the empty tube, the recorded wall temperature is that corresponding to the temperature of the stationary gas film in contact with the wall, but is considerably higher than the average gas temperature throughout the reaction chamber.

It will further be observed that the concentration of propylene in the product obtained in the empty tube is only about one-half of the concentration of propylene in the product from the baffled tube. The high percentage of hydrogen and the formation of liquids in the empty tube are due to the polymerization of a portion of the olefines formed.

The same effect is still more strikingly shown in experiments in which a larger furnace and higher gas rate were used.

The following two experiments were carried out in a furnace 80 cm. long and 2.5 cm. in diameter. It consisted of two 40-cm. sections electrically heated by means of nichrome windings, the heating of each section being controlled independently. Baffles (2.2 cm.) were used in the first or pre-heating section in both experiments and the temperature of the outlet end of this section was maintained at the same point in both cases. Consequently in each experiment the gas entered the reacting section at the same temperature.

In the first experiment no baffles were used in the 40-cm. reactor, whilst in the second experiment 2.2-cm. baffles were used in this section. The results are shown in Table VII.

TABLE VII

TEMPERATURE REQUIRED TO PRODUCE EQUAL EXPANSIONS IN EMPTY AND IN BAFFLED TUBES

—	Empty tube	Baffled tube	—	Empty tube	Baffled tube
Gas rate, l/hr.	801	801	Analysis of gaseous product, % by volume		
Wall temp., preheater, °C.	949	949	$C_2H_2$	2.0	1.1
Wall temp., reactor, °C.	1041	946	$C_2H_4$	25.7	26.1
Expansion, %	84	88	$C_2H_6$	8.1	11.7
Gm. olefines per hr.	697	821	$H_2$	19.0	17.5
By-products	Liquids and tar in considerable quantity	None			

It will be observed that the difference in wall temperature amounts to 95°C. Since in both experiments the gas entered the reactor at the same temperature, it is believed that these results show conclusively that the temperature distribution in the reactor in the absence of baffles is not uniform and that actually only a portion of the gas is subjected to the high temperature near the wall of the tube; otherwise the amount of reaction in the unbaffled tube would be greater, whilst actually the amount of reaction in the latter tube was less than in the baffled tube at the lower temperature.

### (b) EFFECT OF BAFFLES ON THE COURSE OF THE REACTION

The following two experiments illustrate particularly the effect of turbulence in the gas flow on the course of the reaction when propane is pyrolyzed:—

On passing the gas, at 400 litres per hour through an unbaffled reaction tube and through a baffled tube, both tubes being 40 cm. in length and 2.5 cm. in diameter, it was found that on gradually raising the temperature, a point was reached at which the appearance of a mist in the exit gas indicated the formation of liquid by-products. The expansion observed at this point is referred to as the critical expansion. The results obtained are shown in Table VIII.

TABLE VIII  
CRITICAL EXPANSIONS IN EMPTY AND IN BAFFLED TUBES

—	Empty tube	Baffled tube	—	Empty tube	Baffled tube
Temp., °C.	920	981	Analysis of gaseous product, % by volume		
Gas rate, l/hr.	399.1	395.0			
Critical expansion, %	35.1	91.2			
Gm. olefines per hr.	180.5	413.5			
			C <sub>3</sub> H <sub>2</sub>	0.6	1.0
			C <sub>3</sub> H <sub>4</sub>	16.4	27.7
			C <sub>3</sub> H <sub>6</sub>	6.9	10.4
			H <sub>2</sub>	12.2	17.2

In the empty tube, the polymerization of olefines to liquids began when the wall temperature reached 920°C. at which point the critical expansion was only 35.1%, which corresponds to an olefine content of 23.3%, as shown by the analysis of the products.

In the baffled tube however, at the same gas rate, it was possible to raise the tube wall temperature to 981°C. before there was any evidence of side reactions, the corresponding expansion being 91.2% and the concentration of olefines in the exit gas being 38.1% by volume. Under the above conditions the percentage conversion based on theory of entering propane to olefines, as calculated from the above figures, was 31.5% when an empty tube was used and 72.9% in the case of the baffled tube.

## (c) EFFECT OF BAFFLE SPACING AND BAFFLE DIAMETER ON THE REACTION

As we have seen, the pressure drop through a baffled tube is actually due to turbulence and not to the impact of the gas stream on the baffles since, within limits, the pressure drop is independent of the number of baffles used. It was consequently thought of interest to determine the effect of baffle spacing on the conversion of propane to olefines in order to find out whether the same effect would be observed as regards the course of the reaction.

The results shown in Table IX were obtained with a 40-cm. tube of 2.5 cm. diameter, the baffle diameter being 2.25 cm.

TABLE IX  
RELATION BETWEEN BAFFLE SPACING AND CRITICAL EXPANSION

Expt. No.	Baffle spacing, cm.	Rate, l/hr.	Temp., °C.	Critical expansion, %	Analysis of gaseous products, % by volume				n for paraffin residue	Olefines produced, gm./hr.
					C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	H <sub>2</sub>		
1	1.2	395	967	92.7	1.4	27.7	9.5	18.2	1.24	399
2	1.6	396	971	89.7	1.5	27.7	10.1	17.5	1.56	402
3	2.8	398.1	970	87.0	1.6	26.9	10.5	17.5	1.61	397
4	4.0	397.3	990	83.5	1.3	27.3	9.9	17.5	—	384
5	6.0	397.3	987	82.0	1.3	27.0	9.1	17.8	—	354
No baffles		399.1	920	35.1	0.6	16.4	6.9	12.2	—	180

It will be seen that the effect of baffle spacing on the amount of olefines produced per hour begins to be appreciable when the baffle spacing is increased from 1.2 to 4.0 cm., but even at a baffle spacing of 6.0 cm., when the number of baffles is only one-fifth of the number when a 1.2 cm. spacing is used, there is no very marked drop in the amount of olefines produced. In the absence of baffles, however, there is a 50% drop in the amount of olefines produced:

In the above experiments, the temperature was raised until the appearance of a mist in the off-gas indicated the formation of by-products resulting from the polymerization of a portion of the olefines.

The effect of turbulence on the course of the reaction is further illustrated by the results obtained in experiments with ethane, propane and *n*-butane. The following experiments were carried out in a furnace consisting of a glazed quartz tube 2.0 cm. in diameter and having a heated length of 18.5 cm. The wall thickness of the tube was 3 mm. Two series of experiments were carried out with and without baffles respectively, to determine the effect of turbulence on the reaction. In the experiments with baffles, mica disks of 1.7 cm. diameter were used and were mounted on a 0.3 cm. diameter quartz rod, the spacing of the baffles being 1.8 cm. Temperature measurements were made by means of a thermocouple situated 4 cm. from the exit end of the heated section. The results are shown in Table X. Using the empty tube, it was found that the formation of mist began around 900°C. and above this temperature liquids were produced in increasingly large amounts.

TABLE X  
PYROLYSIS OF ETHANE IN EMPTY AND IN BAFFLED TUBES

Expt. No.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	Higher* olefines	H <sub>2</sub>	
Empty tube								
6	945	206.3	35.8	0.8	27.6	0.4	29.2	99.4
7	995	208.8	52.0	1.4	26.9	0.9	33.9	113.8
8	1045	208.0	60.8	3.0	24.0	0.9	38.9	108.0
Baffled tube								
9	950	203.8	52.5	0.4	32.6	2.3	35.2	143.7
10	960	208.8	58.1	1.1	33.8	0.2	37.6	141.2
11	980	207.1	67.6	2.1	32.7	1.1	41.2	151.3

\*Calculated as butylenes.

TABLE XI  
PYROLYSIS OF PROPANE IN EMPTY AND IN BAFFLED TUBES

Expt. No.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	H <sub>2</sub>	
Empty tube								
12	902	205.0	35.5	0.6	15.4	6.9	10.1	89.5
13	950	209.7	48.7	0.8	17.9	6.6	12.8	106.9
14	1000	209.7	60.8	1.8	19.0	6.3	16.6	119.9
15	1050	207.2	78.3	2.6	19.2	4.8	21.6	122.1
Baffled tube								
16	860	210.5	54.4	0.5	19.2	12.1	14.2	151.7
17	900	205.4	73.5	0.9	23.1	11.9	16.1	182.4
18	950	206.3	99.6	2.6	27.8	8.8	18.9	211.3

NOTE:—Length of tube, 18.5 cm.; diameter of tube, 2.0 cm.

In Experiment 8, in addition to a large amount of mist and tar, there was a considerable deposition of carbon in the reaction tube. The percentage of ethylene in the off-gas decreased with increasing temperature whilst that of hydrogen increased, indicating loss of ethylene by side reactions.

In Experiment 9 there was no evidence of side reactions and even at 960°C. (Expt. 10) there was only a slight amount of mist in the exit gas. Hence the critical expansion can be taken as 58.1%.

The effect of the baffles is indicated by a comparison of the olefine yields in grams per hour. In the unbaffled tube the maximum production was 113.8 gm. per hr. in Experiment 7, whilst the use of baffles resulted in a yield of 151.3 gm. per hr. (Expt. 11) at a somewhat lower tube wall temperature.

Results very similar to those shown above were obtained when the pyrolysis of propane was carried out under conditions of streamline and turbulent flow. The furnace was used in these experiments as in the case of ethane. The data obtained are recorded in Table XI.

Two experiments have been carried out in a furnace 20 cm. long and 2.7 cm. in diameter. The increase in the yield of olefines due to turbulence seems to be somewhat more marked at the temperature and gas rate used in these experiments, the amount of olefines produced per hour being almost doubled when the reaction was carried out in the baffled tube (Expt. 19) as compared to the empty tube (Expt. 20). The results are given in Table XII.

TABLE XII

PYROLYSIS OF PROPANE IN EMPTY AND IN BAFFLED TUBES AT HIGH TEMPERATURES

Expt. No.	Type of tube	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
					C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	C <sub>3</sub> H <sub>8</sub>	H <sub>2</sub>	
19	Empty	1032	412	30.5	1.1	16.1	5.2	12.0	161
20	Baffled	1035	404	58.3	0.6	19.1	12.6	14.3	304

NOTE:—Length of tube, 20 cm.; diameter of tube, 2.7 cm.

With *n*-butane, which on thermal decomposition gives ethylene and propylene, the absence of side reactions under conditions of turbulent flow is very well illustrated by comparing the percentages of propylene in the exit gas when using the baffled tube with the concentration of this olefine when using the empty tube.

The experiments were carried out with the 18.5 by 2.0 cm. furnace as in the case of ethane and propane and the data obtained are shown in Table XIII. It will be seen that in the absence of baffles the percentage of propylene in the off-gas never exceeded 10.9% (Expt. 22) and in this experiment the formation of a mist indicated a certain amount of side reactions. With the baffled tube there was no mist formation until an expansion of 67.6% had been reached (Expt. 26) and in this experiment the percentage of propylene in the exit gas was 17.0. It will be observed that under these conditions the amount of olefines produced at a given tube wall temperature was very nearly doubled when baffles were used.



TABLE XIII

PYROLYSIS OF *n*-BUTANE IN EMPTY AND IN BAFFLED TUBES

Expt. No.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.					Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
Empty tube									
21	852	203 8	24 2	0 4	8 9	10 2	1 4	6 2	85 4
22	910	204 6	45 2	0 6	12 4	10 9	1 7	8 1	94 7
23	950	202 1	63 0	0 9	15 8	10 5	2 7	10 7	152 1
23A	990	204 6	78 4	1 6	19 1	10 0	2 2	13 0	175 7
Baffled tube									
24	840	205 4	43 1	0 4	11 8	13 9	2 9	8 1	141 5
25	868	202 1	56 3	0 5	13 7	15 1	2 6	9 8	164 2
26	895	203 8	67 6	0 8	14 9	17 0	1 8	11 2	189 2
27	922	203 8	83 3	1 3	18 3	15 0	2 1	12 6	210 4

## Experiments in Baffled Tubes

## (1) Ethane

In addition to the experiments recorded above, the following experiments were carried out to determine the effect of varying the time of contact and temperature on the yield of olefines in a baffled tube.

The first experiment was carried out in a 20-cm. furnace with a tube of 2.5 cm. diameter. The next five experiments were carried out in a 40-cm. furnace with tubes of 2.7 cm. and 2.5 cm. diameter, and the last two experiments in an 80-cm. furnace with a tube of 2.5 cm. diameter. The data are shown in Table XIV.

TABLE XIV

EFFECT OF TEMPERATURE AND TIME OF CONTACT ON THE PYROLYSIS OF ETHANE IN BAFFLED TUBES

Expt. No.	Length of tube, cm.	Diam. of tube, cm.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.			Olefines produced, gm./hr.
						C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	H <sub>2</sub>	
28	20		1091	400.6	66.5	1.0	33.8	35.6	282
29	40	2.7	951	408.0	57.8	0.5	34.7	35.7	280
30	40	2.7	973	406.0	69.8	0.9	35.0	39.6	302
31	40	2.5	933	385.4	60.5	0.3	32.4	35.2	279
32	40	2.5	965	409.2	70.3	0.7	33.4	37.4	308
33	40	2.5	995	397.3	79.5	1.6	32.9	38.8	300
34	80	2.5	916	807.0	46.8	0.3	31.2	31.1	483
35	80	2.5	940	842.0	59.0	0.4	33.1	34.2	577

The absence of side reactions in the above experiments is very well shown by the close agreement between the concentrations of ethylene and hydrogen in the gas produced.

(2) *Propane*

Similar experiments were carried out with propane. As indicated in Table XV, the first experiment was carried out with a 20-cm. furnace, the others with a 40-cm. or 80-cm. furnace. In all experiments the diameter of the tube was 2.5 cm.

TABLE XV  
EFFECT OF TEMPERATURE AND TIME OF CONTACT ON THE PYROLYSIS OF PROPANE  
IN BAFFLED TUBES

Expt. No.	Length of tube, cm.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
					C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub>	
36	20	1087	402.5	76.4	1.4	22.6	13.3	15.4	377
37	40	918	396.5	58.9	0.3	20.7	11.7	13.8	301
38	40	960	396.5	80.7	0.5	24.8	11.0	15.4	370
39	40	981	395.0	91.2	1.0	27.7	10.4	17.2	409
40	40	992	609	63.9	0.8	22.6	11.3	14.8	495
41	40	1020	616	75.0	1.2	25.4	11.2	16.5	572
42	40	992	707	52.0	0.3	19.3	11.2	13.7	488
43	40	1020	705	61.2	0.5	21.5	11.5	15.0	550
44	80	885	417	85.0	1.4	26.8	11.6	17.8	425
45	80	900	510	85.1	1.2	26.9	11.3	14.7	518
46	80	919	607	89.0	1.4	26.2	12.5	17.3	646
47	80	934	708	89.8	1.0	26.2	11.9	17.5	746
48	80	947	801	88.1	1.1	26.1	11.7	17.5	823
49	80	955	700	101.0	1.6	28.6	10.1	17.9	770
50	80	844	807	40.0	0.1	14.0	9.9	11.1	422
51	80	872	801	51.6	0.4	17.6	10.5	13.6	507
52	80	896	807	63.2	0.4	20.0	12.5	14.5	639

The temperature required to convert about 70% of the entering propane to olefines varies with the length of the reaction tube as shown in Table XVI.

TABLE XVI  
RELATION BETWEEN LENGTH OF TUBE AND TEMPERATURE

Expt. No.	Length of tube, cm.	Temp., °C.
36	20	1087
38	40	960
44	80	885

The temperatures were recorded by means of a thermocouple placed at a distance of 6 to 10 cm. from the exit end of the heated section.

The thermocouple was placed against the outside surface of the quartz tube and between two windings, the thermocouple being held in place by means of alundum cement, the entire furnace being well insulated with asbestos.

It will be seen that the temperature at which a high rate of conversion of paraffins to olefines takes place has been considerably lowered by the use of baffles in the reaction tube. In Experiment 44, for instance, at a gas rate of 417 litres of propane per hour, over 70% of the entering propane was converted

to olefines at 885°C. This suggested the possibility that heat resistant alloy steel tubes could be used for carrying out this reaction. This phase of the investigation will be the subject of a later communication.

### (3) *n*-Butane

A number of experiments were carried out with *n*-butane, some in the 40 by 2.5 cm. tube and the others in the 80 by 2.5 cm. tube. It will be observed that the temperature required for a given conversion of the paraffin to olefines was appreciably lower than in the case of either ethane or propane. The results of the experiments with *n*-butane are given in Table XVII.

TABLE XVII  
EFFECT OF TEMPERATURE AND TIME OF CONTACT ON THE PYROLYSIS OF *n*-BUTANE IN BAFFLED TUBES

Expt. No.	Length of tube, cm.	Temp., °C.	Gas rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.					Olefines produced, gm./hr.
					C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
53	40	881	396.5	61.9	0.2	14.5	16.8	5.0	11.4	399
54	40	913	399.0	80.8	0.7	16.5	18.4	4.3	12.7	473
55	40	940	397.5	97.6	1.1	18.7	18.4	3.1	13.6	515
56	80	827	808	41.0	0.2	9.2	12.6	4.4	7.7	545
57	80	858	812	58.0	0.2	11.5	14.7	5.0	9.4	700
58	80	898	808	73.7	0.6	14.0	16.3	5.8	9.7	879
59	80	915	808	88.1	0.6	15.8	17.3	5.3	11.7	995

### (3) *Isobutane*

The two series of experiments recorded in Table XVIII were carried out in the 20-cm. and the 40-cm. furnace respectively.

There are indications that the isobutane used in these experiments contained considerable amounts of *n*-butane, possibly up to 40%. Consequently the above figures cannot be regarded as truly representing the results obtainable by the pyrolysis of pure isobutane in the baffled tube. It is expected that with the pure paraffin, the concentration of propylene in the product would be appreciably higher, especially at the lower temperatures.

TABLE XVIII  
EFFECT OF TEMPERATURE ON PYROLYSIS OF ISOBUTANE IN BAFFLED TUBES

Expt. No.	Length of furnace, cm.	Diam., cm.	Temp., °C.	Rate, l/hr.	Expansion, %	Analysis of gaseous products, % by vol.					Olefines produced, gm./hr.
						C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
60	40	2.7	1089	401.5	75	.08	15.9	15.7	4.7	13.8	397
61	40	2.7	1100	406.0	81	1.2	17.1	15.2	4.4	14.5	413
62	40	2.5	942	398	124	2.0	21.3	13.7	3.3	16.5	492
63	40	2.5	947	401.5	121	2.5	20.2	14.9	3.8	16.1	516
64	40	2.5	913	400.5	102	2.3	17.2	17.0	4.8	14.8	490

## EXPERIMENTS WITH LIQUID PARAFFINS

Several experiments have been carried out in baffled reaction tubes on the pyrolysis of the three lower liquid paraffins. In all of these experiments, except in No. 75, a furnace 20 cm. long by 2.7 cm. diameter has been used, the latter experiment being carried out in a 40-cm. furnace, with a tube 2.5 cm. in diameter.

These experiments were characterized by the absence of tar formation and carbon deposition, in spite of the high temperatures used, and the high concentration of olefines in the products obtained.

(5) *Pentane*

The two last experiments of the pentane series (Table XIX) were carried out with pure isopentane, the others with *n*-pentane. It will be observed that there is an appreciable increase in the concentration of the butylenes when the iso-compound is used.

TABLE XIX  
PYROLYSIS OF *n*-PENTANE AND ISOPENTANE IN BAFFLED TUBE

Expt. No.	Temp., °C.	Liquid rate, gm./hr.	Gas rate out., l/hr.	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
65	1015	1206	580	20.7	18.5	—	8.1	351
66	1070	1404	615	24.3	17.6	—	8.9	392
67	1075	1158	698	24.0	20.3	1.1	9.5	475
68	1071	1080	667	26.7	20.3	1.5	12.1	475
69	1072	1105	645	20.7	19.9	3.5	11.1	465
70	1075	1080	668	19.4	18.9	4.5	11.3	474

(6) *Hexane*

The hexane used had the following composition:—2-methyl pentane, 40; 3-methyl pentane, 20; *n*-hexane, 30; pentanes and heptanes, 10%.

All of the experiments with hexane (Table XX) were carried out in the 20-cm. furnace with a tube 2.7 cm. in diameter, except in the case of the last experiment in which the furnace used was 40 cm. by 2.5 cm. diameter.

TABLE XX  
PYROLYSIS OF MIXED HEXANES IN BAFFLED TUBE

Expt. No.	Temp., °C.	Liquid rate, gm./hr.	Gas rate out., l/hr.	Analysis of gaseous products, % by vol.				Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
71	1032	910	555	27.1	23.1	3.3	10.4	480
72	1043	1310	527	26.8	27.9	—	11.0	451
73	1082	864	617	28.6	20.4	—	11.8	448
74	1081	872	618	30.0	21.6	—	11.5	442
75	952	905	694	29.0	19.7	2.1	12.3	544

## (7) Heptane

The heptane used was of the commercial grade. These experiments were all carried out in the 20-cm. furnace with a tube of 2.7 cm. diameter. The results in Table XXI show that the pyrolysis of the lower liquid paraffins can be controlled so as to yield gaseous products containing between 50 and 55% by volume of olefines. The olefines consisted of approximately equal amounts of ethylene and propylene.

TABLE XXI  
PYROLYSIS OF COMMERCIAL HEPTANE FRACTION IN BAFFLED TUBE

Expt. No.	Temp., °C.	Liquid rate, gm./hr.	Gas rate out, l/hr.	Analysis of gaseous products, % by vol				Olefines produced, gm./hr.
				C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>8</sub>	H <sub>2</sub>	
76	1043	1060	487	27.6	24.2	2.9	10.8	426
77	1075	1042	562	29.0	24.5	1.5	11.4	483
78	1093	767	596	31.6	12.5	0.6	16.8	384

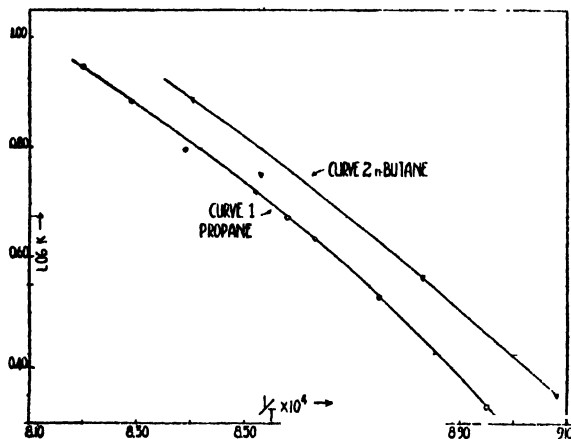


Fig. 4. Variation of  $\log K$  with the reciprocal of the absolute temperature.

The results obtained with the lower liquid paraffins indicate that probably similar results could be obtained by the pyrolysis of the higher boiling members of the series under the same conditions. It is proposed to investigate this phase of the problem in the near future.

*Propane Experiments*

Increasing the temperature at constant gas rate. The results obtained in the propane experiments 50, 51, 53 and 48 are given in Table XXII. The values of  $\log K$  and  $1/T$  calculated from these experiments have been plotted in Curve 1 (circles). Fig. 4.

TABLE XXII

VALUE OF  $K$  FOR PROPANE AT DIFFERENT TEMPERATURES AS CALCULATED FROM EQUATION (1)

Expt. No.	$T$	$a-x$	$t$	$K$	$\log K$	$1/T \times 10^4$
50	1117	66.1	0.194	2.14	0.3303	8.95
51	1143	54.2	.182	3.38	.5289	8.75
53	1167	45.3	.169	4.71	.6730	8.58
48	1220	28.7	.145	8.85	.9469	8.20

*Increasing both the temperature and the gas rate.* In the following experiments, the rate was gradually increased from 400 to 800 litres per hour, the temperature being so regulated that the amount of decomposition was about the same in each experiment. The results obtained in this series of experiments are shown in Table XXIII, and the values of  $\log K$  plotted against the values of  $1/T$ , in Curve 1, Fig. 4 (squares).

TABLE XXIII

VARIATION OF  $K$  WITH TEMPERATURE FOR EQUAL VALUES OF  $(a-x)$ 

Expt. No.	$T$	$a-x$	$t$	$K$	$\log K$	$1/T \times 10^4$
44	1158	26.8	0.310	4.13	0.6335	8.63
45	1173	28.0	.244	5.26	.7210	8.52
46	1192	25.4	.205	6.26	.7966	8.39
47	1207	27.6	.167	7.68	.8859	8.29
48	1220	28.7	.145	8.85	.9470	8.20
		Mean 27.7				

*Temperature coefficient of  $K$  for propane.* The velocity constant of the decomposition of propane into ethylene and propylene is increased 1.231 times per  $10^\circ\text{C}.$  rise in temperature at  $845^\circ$ , and 1.095 times per  $10^\circ$  rise at  $940^\circ\text{C}.$

### Butane Experiments

*Increasing temperature.* The results obtained in a series of butane experiments are given in Table XXIV. The calculated values of  $\log K$  and  $1/T$  are plotted in Curve 2, Fig. 4 (triangles).

TABLE XXIV

VALUE OF  $K$  FOR BUTANE AT DIFFERENT TEMPERATURES AS CALCULATED FROM EQUATION (1)

Expt. No.	$T$	$a-x$	$t$	$K$	$\log K$	$1/T \times 10^4$
56	1101	64.5	0.195	2.25	0.3522	9.08
57	1131	52.6	.175	3.66	.5635	8.83
58	1171	40.2	.161	5.65	.7521	8.53
59	1191	31.4	.150	7.70	.8865	8.40

The temperature coefficient of  $K$  for butane is very closely the same as for propane between  $845^\circ$  and  $940^\circ\text{C}.$

### Velocity Constants and Temperature Coefficients of the Rate of Conversion of Propane and *n*-Butane to Olefines

It was thought of interest to calculate the velocity constants and the temperature coefficients of the reaction rates from the results obtained in the baffled 80-cm. reaction tube, since a considerably higher amount of decomposition can be obtained under these conditions without appreciable side reactions than with open tubes.

R. N. Pease and E. S. Durgan (11) have shown that the decomposition of propane and *n*-butane is unimolecular and homogeneous and consequently the velocity constant can be calculated by the following formula,

$$K = \frac{2}{t} \log \frac{a}{a-x}, \quad (1)$$

where  $K$  is the velocity constant of formation of ethylene and propylene;  $t$ , time of contact in seconds;  $a$ , the amount of hydrocarbon at the start; and  $a-x$ , the amount of hydrocarbon remaining after time  $t$ .

With regard to the estimation of the time of contact, the fact that the two halves of the reaction tube were heated separately made it possible to bring the gas up to reaction temperature in the first half of the tube, and to maintain a uniform temperature in the second half. The volume of the reaction chamber was consequently taken as half of the total volume of the tube, that is, 202 cc. The volume of the gas passing through the tube was of course corrected for thermal expansion, and for the expansion due to the reaction.

#### *Variation of $K$ and $E$ with Temperature*

It will be observed that the change in slope of the curves indicates an abnormal falling-off in the value of  $K$  as the temperature is increased. The abnormal decrease in the velocity constant  $K$  observed in these experiments may be due to the fact that at the high temperatures used the rate of the reverse reaction, in particular the rehydrogenation of the olefines, begins to be appreciable compared with the forward reaction. Pease and Durgan (11), observed an abnormal decrease in the rate of decomposition of propane and *n*-butane at temperatures as low as 625°C. at a time of contact of 12 sec. This they believe was due to the rehydrogenation of some of the olefines. It should be noted, however, that although *n*-butane gives a product in which the concentration of hydrogen is considerably lower than in the case of propane, yet its rate of decomposition falls off to about the same extent as the rate of decomposition of the latter.

The heat of activation of the decomposition of propane and *n*-butane has been calculated at 850°C. from the slopes of the two curves, which are almost identical at this point. The value found was 45,000 calories. This is considerably lower than the value calculated by Pease and Durgan for the same reaction, their value being 63,000 calories at 625–630°C., but this difference of course was to be expected in view of the very marked decrease in the temperature coefficient with rising temperature, in the range investigated in the present experiments.

### Thermal Efficiency of the Baffled Tube

It has already been pointed out that one would expect a definite increase in the rate of heat transfer from tube wall to gas as the gas rate in the baffled tube is increased. Partly in order to test this out and also to determine the relation between the amount of heat required to bring the paraffin up to reaction temperature and that used up in the reaction, a heat balance was calculated for several of the above experiments.

The following heats of reaction were used in the calculation. These figures were calculated from data in Landolt and International Critical Tables, or estimated when heat of combustion data were lacking.

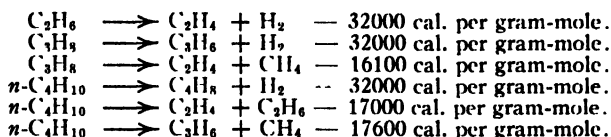


Table XXV shows the thermal efficiency of the 80-cm. tube in the decomposition of ethane, propane and *n*-butane. In Table XXV the letters have the following significance:— $W$ =total heat supplied, in kwh.;  $H$ =heat absorbed in formation of the amount of olefines found in product of reaction, in kilocalories;  $W_1=H$ , in kwh.;  $W_2$ =heat lost through insulation of furnace;  $W_3=W-(W_1+W_2)$ =heat taken up by the gas when temperature is raised to  $T$ ;  $T.E$ =thermal efficiency,  $=\frac{W_1}{W} \times 100$ .

The falling-off in the thermal efficiency of the tube with increasing molecular weight of the hydrocarbon may be explained by the increasing amount of heat required to raise the gas up to reaction temperature, as can be seen by comparing the values of  $W_1$ ,  $W_2$  and  $W_3$  in Table XXV. In the ethane experiment, for instance, the heat absorbed in the reaction ( $W_1$ ) is nearly equal to the heat required to heat the gas to reacting temperature ( $W_3$ ). With propane  $W_1$  is about one-half of  $W_3$ , whilst for *n*-butane  $W_1$  is about one-third of  $W_3$ .

TABLE XXV

THE THERMAL EFFICIENCY OF THE 80-CM. TUBE IN THE PYROLYSIS OF ETHANE, PROPANE AND BUTANE

Expt. No.	Gas	Temp., °C.	W, kwh.	Olefines formed, gm.	H, kilocal.	W <sub>1</sub>	W <sub>2</sub>	W <sub>3</sub>	T.E., %
						kwh.			
35	Ethane	940	2.377	C <sub>2</sub> H <sub>4</sub> , 577	660	0.767	0.735	0.855	32.4
48	Propane	947	2.682	C <sub>3</sub> H <sub>6</sub> , 450 C <sub>2</sub> H <sub>4</sub> , 336	515	.598	.755	1.329	22.4
59	<i>n</i> -Butane	918	2.946	C <sub>4</sub> H <sub>8</sub> , 297 C <sub>3</sub> H <sub>6</sub> , 492 C <sub>2</sub> H <sub>4</sub> , 201	501	.581	.695	1.670	19.7



*Current Consumption in the Production of Olefines in the 80-cm. Furnace*

Some values for current consumption in the production of olefines by the pyrolysis of ethane, propane and *n*-butane in an 80-cm. baffled tube are given in Table XXVI.

TABLE XXVI

CURRENT CONSUMPTION IN THE PYROLYSIS OF ETHANE, PROPANE AND *n*-BUTANE IN BAFFLED TUBES

Expt. No.	Hydro-carbon	Gas rate, l/hr.	Temp., °C.	Current, watts	Olefines produced gm./hr.	Kwh. /lb. olefines
47	Propane	708	934	2429	746	.1 48
48	Propane	801	947	2690	823	1 48
34	Ethane	807	916	2080	483	1 95
35	Ethane	842	938	2377	577	1 86
58	<i>n</i> -Butane	808	898	2744	879	1 42
59	<i>n</i> -Butane	808	913	2946	995	1.34

The current consumption for the production of olefines in a baffled tube is remarkably low, especially when a comparison is made with the current consumption for the commercial production of acetylene by the carbide process in which 4.5 kwh. are required per pound of acetylene produced. This is interesting in view of the fact that acetylene has been considered as a possible material for the production of ethylene (7, 15).

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## SOME MEASUREMENTS OF THE ELECTROSTATIC PROPERTIES OF PHOTOGRAPHIC FILMS<sup>1</sup>

BY D. C. ROSE<sup>2</sup>

### Abstract

This paper deals with experiments performed to determine the electrostatic properties of celluloid camera films. The information derived was used in attempts to eliminate marks due to static electricity on film used in aerial photography.

Camera films were rubbed with wool or brass and the resulting electrostatic effects studied. The required atmospheric conditions were reproduced by carrying out the experiments in a tank in which pressure, temperature and humidity could be controlled independently. It was found that the charging effect was more pronounced in a moist than a dry atmosphere.

Methods of eliminating the trouble caused by static electricity in aerial photography are discussed.

A study has been made of the amount of static electricity generated when pieces of celluloid camera film were rubbed by various materials under different atmospheric conditions. The object of the investigation was to try to eliminate the generation of static electricity in cameras used for aerial photography. The discharge resulting from such static charges marks the film, often so badly that it is useless for mapping or survey work, making it necessary to re-photograph large areas.

The problem of the prevention of marks on films due to electrostatic charging was undertaken from two points of view, first, a study of the conditions under which the film was used in the aerial camera (this involved a study of the atmospheric conditions under which photographic flights were made); and second, a study of the electrostatic charges acquired by celluloid films when subjected to atmospheric and other conditions approximating those under which it is used in practice.

### I. Process of the Charging of the Film as it Moves Through the Camera

Celluloid, which seems to be the only satisfactory material from which photographic films can be made, has the great disadvantage that it acquires electrostatic charges very readily.

<sup>1</sup> Manuscript received July 27, 1933.

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In cameras used for aerial mapping in Canada (size of picture,  $7\frac{1}{4} \times 9\frac{1}{4}$  in.) the film, while exposure is being made, is clamped between a glass focusing plate and a bare metal or felt covered pressure plate. Between exposures the pressure plate is raised slightly so that the film may be rolled on to the next position of exposure. In rolling the film through between the pressure plate and the focusing plate, rubbing on either or both plates cannot be avoided. The process of building up a charge on the surface of the film, sufficiently intense for a spark to jump, depends on so many factors about which so little is known that it is difficult to say exactly where and how the charge is formed. However, an insulating material, *e.g.*, a celluloid film, moving over a piece of felt or metal or a glass plate, such as the pressure plate and focusing plate of the Fairchild aerial camera, creates ideal conditions for the generation of electrostatic charges. Whether or not charges will be generated of course depends somewhat on whether or not the film is charged when it first comes off the roll. If there were no electrostatic charges on the film in the roll, the building up of charges as it moves through the camera would be less likely than if it were charged originally. The experience of the writer is that celluloid is one of the most difficult of insulating materials to keep discharged. Handling of any sort whatever seems to cause it to acquire electrostatic charges. From experience of electrostatics in general one would say that the film should be wound and kept in as moist a condition as possible without spoiling the sensitivity of the emulsion. In fact such a procedure has been recommended. However, the results of recent experiments indicate that the charging effect appears under certain conditions to be greater with the film in a moist atmosphere than in a dry one. The moisture would be expected to cause the static charge to be dissipated or conducted away without a spark while the film is rolled up on the spool but until a more complete investigation is made, this point remains unsettled.

Even if the film is totally discharged when it leaves the roll, it may acquire a charge by rubbing either on the glass plate or on the pressure plate. If it is charged on the roll, the effect of rubbing will likely be magnified. Such charges can easily raise the potential of the film sufficiently high to cause a spark to jump along the surface of the film when it has passed between the pressure plate and the glass plate. This spark appears to jump to the roller or to the metal strip holding the glass plate in place, depending on which side of the film is charged. It jumps along the surface of the film causing the static markings which look somewhat like lightning discharges or a combination of these lightning flash marks and a diffuse black spot (see Plate I, Fig. 1). Fig. 1 is a reproduction of a piece of film used in an aerial camera. The flashes caused by the spark will discharge a band of the film a short distance ahead of the point to which the spark jumps. There will not be another flash until the film has moved on to a point where there is another charge close enough to the roller, or edge of the glass plate, to cause a spark to jump to the metal parts. This accounts for the somewhat regular lines of discharge across the direction of motion of the film. These sparks along the edge of the glass plate may be seen if the film is rapidly rolled through the camera outdoors on a dark dry winter night.

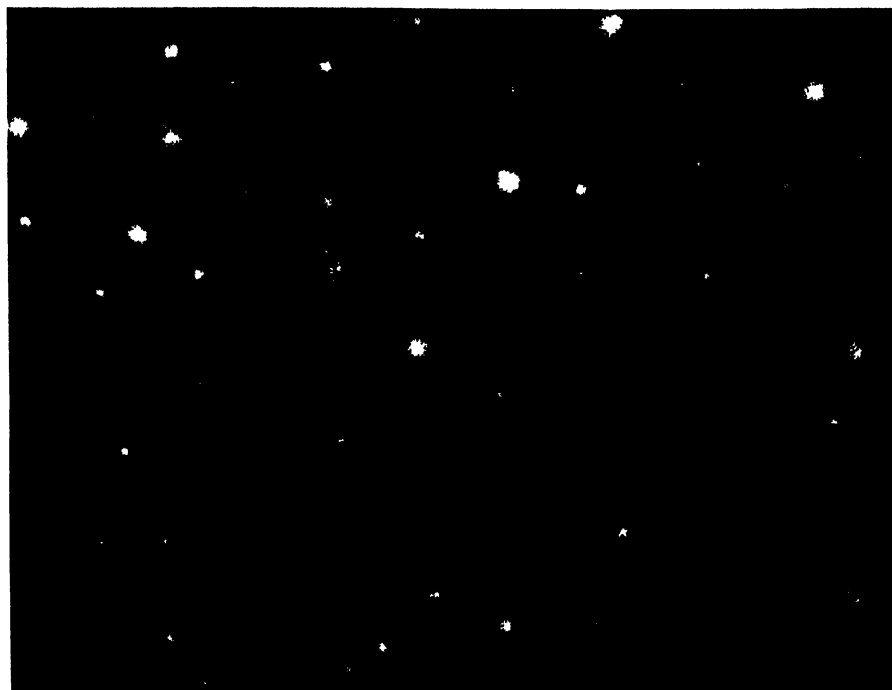


FIG. 2. Static markings on film used in aerial photography.



FIG. 1. Static markings on film used in aerial photography.



Sparks were also seen to jump from one part of the film to another where it comes off the roll. Such sparks would make the round diffuse static marks shown in Plate I, Fig. 2. This type of spot also occurs when sparks jump from the film to other parts of the camera. This also indicates that the film observed by the writer was charged in the roll. From this it need not be inferred that new films are charged in the roll, as the films used for experimental purposes were spoiled films which had been re-rolled a number of times.

## II. Experimental Study of the Charging of Celluloid Film

### *Preliminary Experiments*

In view of the probability of charges being generated as the film passes over the pressure plate, some preliminary tests were made on the relative charging of a piece of film rubbed by different substances. A piece of film, sensitive side down, was clamped on a flat brass plate electrically insulated and connected to an electrostatic voltmeter. The surface of the film was given a number of vigorous rubs with various materials and the deflection of the voltmeter noted when the material was removed. Care was taken that the amount and nature of the rubbing should be approximately the same for each reading. The following materials were used:—celluloid plate, another piece of film, brass plate, wood (white pine), wood-fibre board, wool felt, cardboard, silk, piece of film rubbing with emulsion surface. These materials are listed in such an order that the first gave most charging effect and the last the least. The experiments were performed in the summertime at ordinary room temperatures. The results were fairly consistent when repeated, though the relative positions of some of the materials might change a place or two. The same piece of film was used throughout this experiment. The results of Shaw and Hanstock (6) in experiments on frictional electricity indicate that the electrostatic properties of materials change with continuous rubbing due to distortion of the surface. The film going through the camera would constantly offer a new surface to be rubbed; consequently the result might be different. One would not expect, however, that the order of the above list would change much.

These initial results indicate that the pressure plate should be covered with a piece of undeveloped film with the face or emulsion side exposed. Probably a piece of exposed and developed film would do just as well, as the surface would be black. Such a surface would have to be replaced periodically but should last several months.

To make a more complete study of the electrostatic charging of films under various conditions, it was decided to measure the humidity and other properties of the atmosphere under actual operating conditions. The Royal Canadian Air Force undertook to give the writer a number of flights to measure temperature, relative humidity, and quantity of electric charge in the air under conditions similar to those found in photographic work. Four flights were made, the results of which have been published (4, 5).

It was found that at certain levels humidities up to 80 or 90% might easily be encountered, while the relative humidities at lower and at higher altitudes were considerably less. The height of this level of maximum relative humidity varies with the weather but appeared to be 1,000 to 2,000 ft. above the level at which clouds form when there are such clouds in the sky. During the flights clouds were avoided as much as possible. The difference between the cloud level and the height of the maximum relative humidity level may have been due to the position of the instruments in the slip stream. This has been discussed (4). An incidental result of these observations was the discovery that the hair hygrometer is a very unsatisfactory instrument for aircraft work.

The results of the observations on the electricity or ion content of the air have been published (5). The most striking feature was the fact that a maximum appears in the excess of positive over negative electricity in the air at the same altitudes as the relative humidity maxima (2). The observations on the electric space charge in the atmosphere were taken more because of the excellent opportunity offered in the flights to obtain information on atmospheric electrical effects, than for any significance they might have on the static charging of the film in aerial cameras. It would be contrary to expectations to find that the relatively small electric charge in the atmosphere outside a completely enclosed metallic box like a camera could have an effect on the electrostatic charging that is going on inside the box. This, of course, assumes that the various parts of the complete camera, cone, magazine, and other parts, are not insulated from one another. Further, it would not be expected to matter whether or not the metallic camera itself was electrically connected to the frame of the aeroplane. These parts in cameras examined by the writer were not insulated.

The humidity, on the other hand, and the temperature would be expected to have some effect because, although the camera is effectively a completely enclosed conducting box, it is not air-tight and changes in humidity outside would gradually work into the magazine.

*Apparatus with which Observations were made on the Charging of Films under Conditions Similar to Those Found in Flight*

To reproduce conditions under which effects of static charges appear in photographic flights, a tank was built in which the pressure, temperature and humidity could be controlled. The tank was cylindrical in shape, with flat removable heads. It was lined inside with coils of copper pipe through which cold brine from a refrigerating machine could be pumped.

Attached to one head of the tank was an apparatus in which a piece of film or other material could be clamped to a flat brass plate 3 in. square. This brass plate was supported on insulated posts and a lead was taken out through the head of the tank, to be connected to an electrometer. Above the brass plate was another brass plate parallel to the insulated plate, to which the rubbing material was fastened. This plate was supported by its centre from

a carriage which ran on two rails (see Fig. 3). The support was so arranged that the plate was free to tilt slightly in both directions, so that when pressed down on the lower insulated plate it would adjust itself to make maximum contact with the film. The rails carrying the carriage supporting the rubbing plate could be raised and lowered

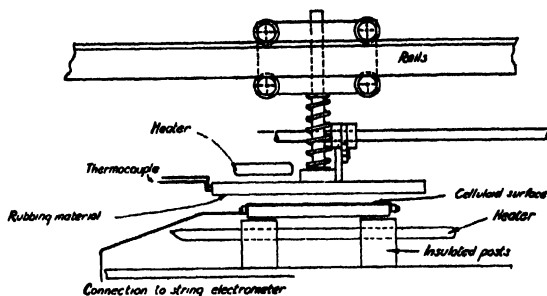


FIG. 3. Apparatus used for rubbing film to produce electrostatic charge.

by a parallelogram link motion controlled from outside the tank. The rubbing plate was moved back and forth by a crank and connecting rod, also controlled from outside the tank. The rubbing plate was pressed onto the insulated plate by a spring which was calibrated, so that the pressure was known. The rubbing plate was  $4\frac{1}{2}$  by 3 in. and the crank motion had a stroke of  $1\frac{1}{2}$  in., so that the insulated plate was completely covered by the rubbing plate at all positions of the crank.

Two electric heaters were placed as shown above the rubbing plate and below the insulated plate. These were used in conjunction with the cooling coils on the tank to produce rapid changes in temperature. The temperature of the rubbing plate was measured by a thermocouple attached to the plate itself, as shown. The temperature of the insulated plate could not very well be measured and still retain the required insulation. However, the heaters and other parts were arranged in so far as possible so that the two plates should warm up and cool down at about the same rate. Possible effects due to temperature differences will be discussed later.

Supported on the other head of the tank was an electric fan to circulate the air, a glass window through which to observe the inside of the tank and a dew point hygrometer for measuring humidities. The hygrometer was hung well out in the tank in front of the window so that the dew point could be observed through the window. The temperatures were measured with thermocouples and a Wolf potentiometer. A platform in the centre of the tank supported a tray of the drying agent or humidifying agent employed.

### *Procedure*

The charging of a piece of film when rubbed by, say, a piece of felt, would be expected to depend on a number of variables. An experimental procedure in studying the charging effect should attempt to fix all but one. The variables concerned in this investigation were temperature, humidity, pressure, rate of rubbing, amount of rubbing, and pressure of rubbing material on the film. The last three, rate of rubbing, the amount of rubbing, and pressure of the rubbing material on the film, were expected to be of secondary importance, as others who have experimented on the electrostatic charges between two substances have found that the charge produced is independent of these variables.



Macky (3) finds that on rubbing such substances as aluminium and sulphur, the charging effect is independent of the normal pressure between the specimens, the velocity of rubbing and the amount of rubbing, provided it is over a certain amount. Macky and others (Kluge (2), Wolf (8), and Jones (1)) using various types of apparatus and materials, usually an insulator and a conductor, show curves, giving amount of charging plotted against amount of rubbing, which indicate that the amount of charging first increases rapidly with the amount of rubbing, then slowly approaches a steady value independent of the amount of rubbing. In the present experiment the same appeared to be the case, though the point was not investigated very thoroughly. The amount of charging of the celluloid when rubbed by brass or felt was found to be independent of the rate of rubbing and of pressure on the plate, in further agreement with other electrostatic experiments.

However, during any run in which temperature, humidity or air pressure variations were being recorded, precautions were taken to turn the crank the same amount, usually one to five turns, depending on conditions, and at the same rate and with the same pressure between the rubbing plates (usually in the neighborhood of 1200 gm. total pressure).

A fibre electrometer was used to measure the voltage produced by the charge on the celluloid. This instrument was found very satisfactory in that its sensitivities could be varied greatly to observe widely different conditions, and the instrument was quite rugged and not easily disturbed.

The procedure for taking an observation was as follows. First, with the rubbing plate lifted about  $\frac{3}{4}$  in. away from the celluloid covered plate, the electrometer was grounded. Then with the electroscope and insulated plate not grounded, the rubbing plate was lowered onto the celluloid. If there were no charge on the material on the rubbing plate or on the celluloid, no deflection of the fibre took place. The crank was then turned the requisite number of times and the rubbing plate was raised from the insulated film-covered plate and the deflection of the electrometer fibre recorded. Then the film was discharged as explained in the following paragraphs. This process was usually repeated three or four times before conditions were changed. The air pressure or whatever variable was being studied was then changed and the process repeated.

### *Discharging the Films*

The obvious way to discharge the surface of the film and rubbing material is to make the air in its neighborhood conducting. In the early experiments with the apparatus, this was done by causing an electric spark to jump from a row of pin points about  $\frac{3}{4}$  in. away from the rubbing plate. This should have caused intense ionization, which should have neutralized the charges on the celluloid film and the rubbing material. Care had to be taken to make the spark jump to metal parts away from the rubbing material (felt) or the celluloid; otherwise it would burn. One series of experiments was spoiled by this taking place. The process of discharging the film was to put the spark on for two or three seconds with the plates separated and grounded.

The spark was effective in discharging the celluloid in experiments in which the celluloid was rubbed with a brass plate, but when a more complex material, like felt, was used the spark would not discharge it. In place of the spark a quartz mercury vapor lamp was tried. This was so arranged that an intense beam of ultra-violet light was thrown between the two plates when separated. This should have caused ionization of the air which would have neutralized the charges on the plates. This was also found to be satisfactory in the case of the brass rubbing plate but not in the case of felt. Apparently the felt became charged throughout its volume and the ionized air in the neighborhood would not penetrate it far enough within reasonable time to discharge it. As a result, instead of discharging the felt its charge was corrected for in plotting the observations as follows: the mercury arc was left on for about 30 sec. with the plates separated and grounded, then turned off, the insulated plate and electrometer ungrounded and the plates closed together. This caused a deflection of the electrometer fibre in an opposite direction to that caused by the charged celluloid. This deflection was taken as the zero point for that particular observation. The rubbing was then carried out and the plates separated, the resulting fibre deflection being read. Whether or not this is a true correction for the residual charge on the felt is uncertain, because the residual charge on the felt would have some influence on the charging effect due to the rubbing. However, qualitative results are all that could be expected in the present experiment and a residual charge on the felt should not affect them much.

## RESULTS OF OBSERVATIONS

### *Variation with Pressure in a Dry Atmosphere*

#### *(a) Felt on Film Back*

To obtain quantitatively repeatable results in measurements of electrostatic charging by rubbing is very difficult and in practice is rarely, if ever, achieved. Even when simple solid substances like aluminium and sulphur (3) are used it is difficult to reproduce a given set of readings quantitatively. This is, no doubt, owing to the fact that the charging is an exchange of electricity between the surfaces involved and even slight rubbing cannot take place without altering the orientation or structure of surface molecules. The experimental procedure is to put the surfaces being studied under a cycle of operations and study the results with repetitions of the cycle under various conditions.

The present experiments were limited to a study of the behavior of pieces of Kodak panchromatic film, taken out of a roll as it would be used in aerial photography, when rubbed by a clean piece of black felt or a clean bare brass plate. The film was cleaned carefully by wiping with cotton wool containing petroleum ether. Only the rubbing on the back or the insensitive side of the film was studied. The usual procedure was to reduce the pressure in the tank by steps taking readings at each step, then increasing and reducing it alternately, keeping all other variables (temperature, humidity, amount and rate of rubbing and pressure on plate) constant. Then this was repeated at

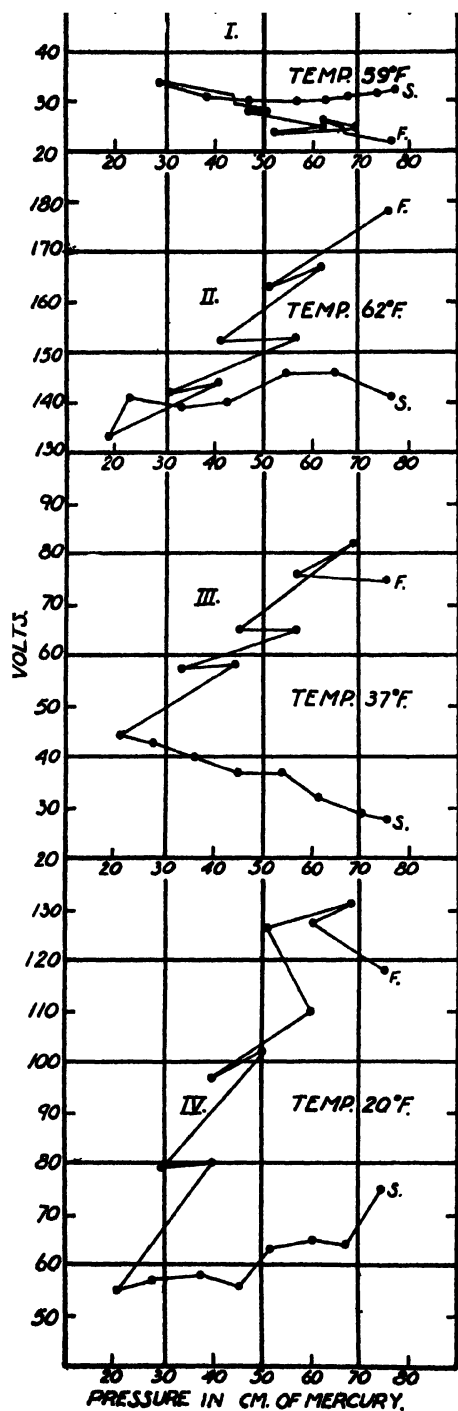


FIG. 4. Variation of electrostatic potentials with pressure when celluloid is rubbed by felt in a dry atmosphere at various temperatures.

different temperatures, and later, temperature runs were taken at constant pressures. Then the whole was repeated at different humidities.

Fig. 4 shows the results plotted in volts to which the plate and electrometer became charged. In the case of Curves I, III and IV, the felt was stretched tightly over the plate and held on by clamps at the ends of the plate. This allowed the felt to rub somewhat on the upper plate and it was thought that this caused some of the large residual charge which was so difficult to remove from the felt; hence in later experiments, of which Curve II and curves in subsequent figures are samples, the felt was stuck to the brass rubbing plate by means of a thin layer of cement made by dissolving collodion in amyl acetate. This did not reduce the residual charge in the felt as was expected. In the case of Curves I, III and IV the amount of rubbing corresponded to one turn of the crank. In the case of Curve II the crank was turned five times. The amounts of charging in the two cases are not comparable because of the different mountings of the felt. Each point on these curves is the average of three or four individual observations. The points in the order in which they were taken are joined by a straight line. In cases where the straight lines overlap (Curve I), the line has been made to deviate slightly to make the figure clearer. The most noticeable feature of these results is the fact that while the pressure is being reduced the charging follows no particular rule, but when the pressure is increased, a greatly increased charging effect usually results. A subsequent reduction in pressure either leaves it constant or reduces it slightly and an increase in pressure

again increases the charging. This is particularly so at low pressures and may reverse near atmospheric pressure. It is also more noticeable at low temperatures than at high, as other curves at temperatures between 60° and 65° F. did not show it, though in Curve II it does appear.

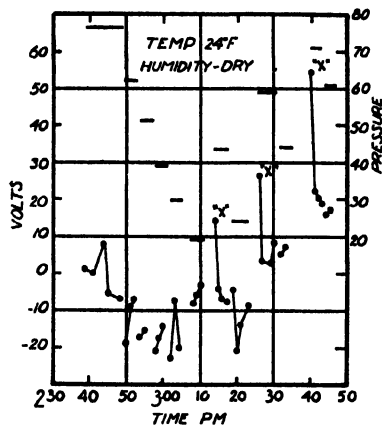


FIG. 5. Variation of electrostatic potential with pressure. The pressure at which each observation was taken is indicated by the heavy bar above it.

slightly and then increased slightly again in a manner not unlike that indicated in the curves in Fig. 4, but when the pressure was increased, a great increase in the charging effect (see point "X") was noted. This effect is analogous to the increased charging with rising pressure in Fig. 4, but is different in that it appeared for the first rub only. An average of the observations at each pressure would not show the effect nearly so pronounced. This effect appeared only at the lower temperatures observed.

A satisfactory explanation of this temporary increase in charging effect has not been found. When it was ascertained that the quantity of charge developed was so sensitive to humidity (see later paragraphs), it was thought that owing to the air from the room being inadequately dried before it entered the tank to raise the pressure, the moisture carried into the tank caused the temporary increase in charging before it was absorbed by the calcium chloride. However,

Later observations, particularly those at low temperatures, indicated a systematic short period change in the charging effect which is indicated in the curve in Fig. 5. In this curve each individual observation is indicated, so that the horizontal axis represents the time at which the observations were taken, the pressure being indicated by the short horizontal bars and the scale on the right side of the figure.

The pressures are indicated in centimetres of mercury. As the pressure was reduced the charging decreased

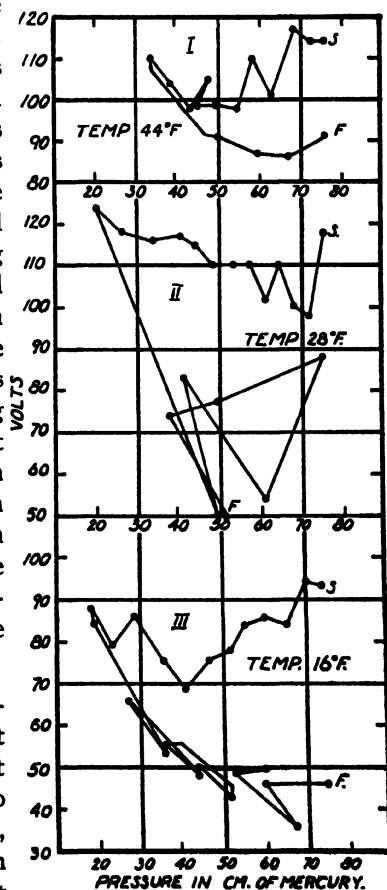


FIG. 6. Variation of electrostatic potentials with pressure when celluloid is rubbed with a bare brass plate in a dry atmosphere at various temperatures. S = beginning of run, F = end of run.

the fact that this temporary increase in the quantity of charge also appeared with increasing pressure when the tank was kept at a humidity of about 60%, indicates that it was probably due to some other adsorption phenomena. This point requires a more detailed investigation before more definite conclusions can be drawn.

(b) *Brass on the Film Back*

Fig. 6 shows the results of experiments in which a smooth, clean, bare, brass plate was used to rub the film in a dry atmosphere at varying pressures and temperatures. The figure indicates that, as before, the variation in the charging effect as the pressure is reduced is not very great and seems to follow no definite rule, but when the pressure is increased a sharp decrease in the charging takes place. This is particularly noticeable at low pressures (about 20 to 50 cm. of mercury) and more noticeable at low temperatures than at high ones. It should be noted here that this change in charging effect with increasing pressure is in the opposite direction to that observed when felt is used as the rubbing material. Also the charge acquired by the celluloid is much greater when rubbed by bare brass than when rubbed by felt.

This is consistent with the early rubbing experiments described in Part I.

*Variation with Temperature in a Dry Atmosphere*

(a) *Felt Rubbing Plate*

The curves shown in Fig. 4 for felt rubbed on celluloid indicate no regular variation of the charging effect for runs at different temperatures, but as stated above they are not entirely comparable as they do not represent a continuous series of runs using the same sample throughout. When all pressure runs at various temperatures are examined together, it is seen that there is a tendency for the mean charging to be lower at lower temperatures. This is also indicated by several runs which were taken at a constant pressure and varying temperature. Fig. 7 shows the results of such a run at a pressure of 60 cm. of mercury. The charging effect rises with the temperature, being several times greater at 40° than at 25° F. A subsequent reduction in temperature does not cause a reduction in charge corresponding quantitatively, but does reduce it considerably. In another run, the day before, starting at 60° F. and decreasing the temperature to about 28° F. the charging effect was reduced from about 60 volts to only four or five volts, the

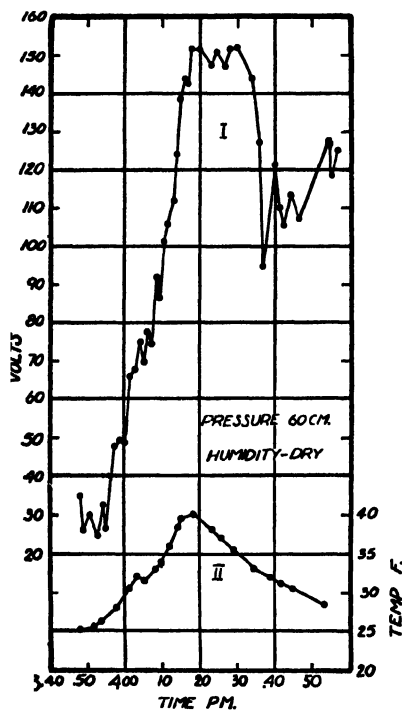


FIG. 7. Variation of electrostatic potential with temperature when celluloid is rubbed with felt in a dry atmosphere. The temperature at which each observation was made is indicated by the part of the temperature curve directly below the point concerned.

reduction varying roughly linearly with temperature. No discontinuity was noted as the temperature passed through freezing point.

(b) Brass Rubbing Plate

When a bare brass rubbing plate was used the variation with temperature, other variables being fixed, was in the same direction as the variation observed when a felt plate was used, but less pronounced.

Variation with Humidity

(a) Felt Rubbing Plate

Time did not permit of an extensive study of the electrostatic charge acquired by celluloid at many different humidities. This was owing to the difficulty of varying the humidity rapidly and accurately in the closed tank, where other variables must be kept under control. The result is that observations were made only in a dry atmosphere (all the results described previous to this were taken in a dry atmosphere) and with a saturated solution of sodium bisulphate in dishes in the tank. In these experiments the fan installed in the tank kept the air in constant circulation, so the humidity should have been uniform. International Critical Tables give the relative humidity

over a saturated solution of  $\text{NaHSO}_4 \cdot \text{H}_2\text{O}$  as 52% at a temperature of 20° C.

The most surprising result of rubbing the film with felt or brass was the greatly increased charging effect. The voltage to which the celluloid became charged when rubbed in this moist atmosphere was usually two or three times that found in a dry atmosphere. Fig. 8 shows pressure curves at 62° and 17° F. The curves are plotted in a similar manner to that of Fig. 5 and should be compared with it. The humidity, according to tables, at a temperature of 62° F. should be about 50%. Actually the dew point hygrometer indicated a slightly higher relative humidity. At the lower temperature the dew point hygrometer indicated a relative humidity of 65%. It will be observed that the increase in

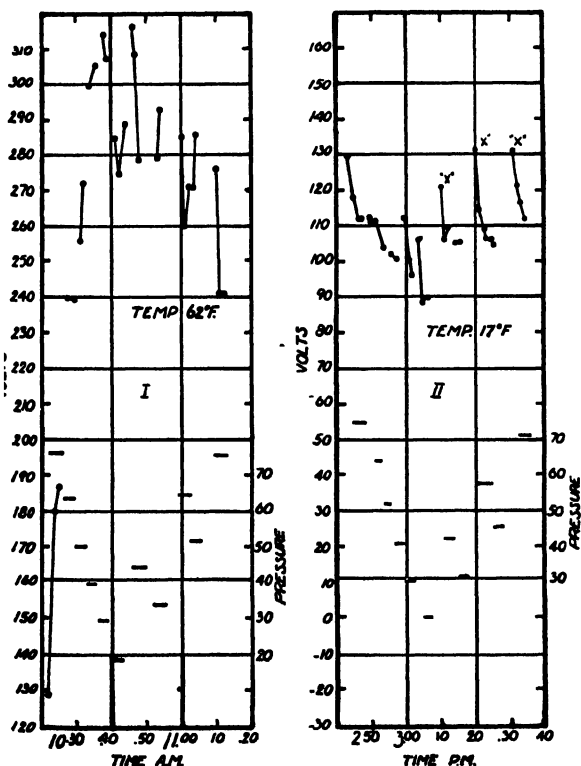


FIG. 8. Variation of electrostatic potential when celluloid is rubbed with felt in a moist atmosphere. The pressure at which each observation was taken is indicated by the heavy bar above or below it.

charge for the first rub after the pressure has been increased is very noticeable at the lower temperature (points marked "x") and not at the higher. This is similar to the observations in a dry atmosphere.

Fig. 9 shows the effect of temperature variation with other variables fixed (pressure 75.4 in case of Curve I and 56.6 cm. for Curve II) and a saturated solution of sodium bisulphate as a humidifier. The humidity, of course, will vary with the temperature and pressure. The curves indicate that the charging effect tends to rise with dropping temperature at pressures near ground pressures (Curve I), while at reduced pressure the charging continues to increase through a rising and falling temperature cycle (Curve II). It would require a considerably more extensive investigation to be sure of the temperature effect.

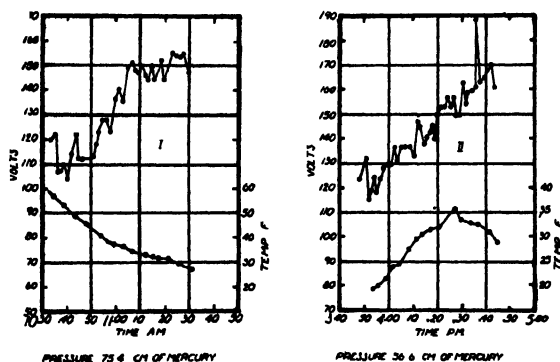


FIG. 9. Variation of electrostatic potential with temperature when celluloid is rubbed with felt in a moist atmosphere. The temperature at which each observation was made is indicated by the part of the temperature curve directly below the point concerned.

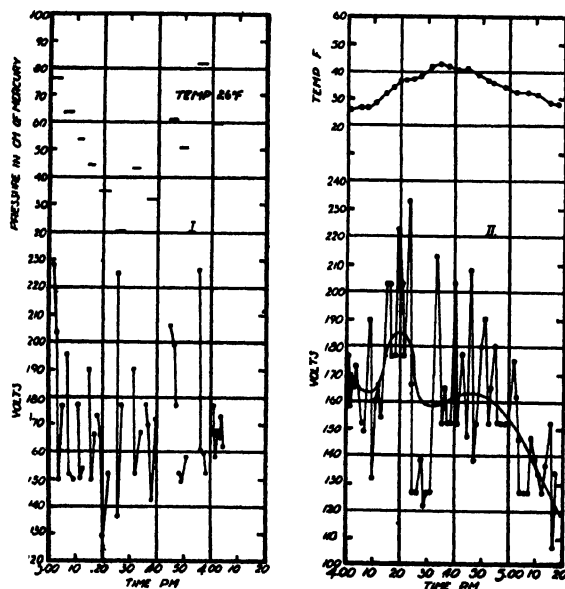


FIG. 10. Variation of electrostatic potential with temperature and pressure when celluloid is rubbed with brass. The pressure or temperature at which each observation was taken is indicated by the heavy pressure bars or the temperature curve immediately above the point concerned.

### (b) Brass Rubbing Plate

When a bare brass rubbing plate is used the results in a humid atmosphere are consistent with those obtained in a dry atmosphere, in that the charging effect is much greater (two or three times) than when the rubbing plate is covered with felt. The amount of charging seems to lessen with reduced temperatures as indicated by a curve not shown, but a temperature cycle (Fig. 10, Curve II) shows no consistent variation as in the case of felt. Fig. 10, Curve I, shows a pressure run at a temperature of 26° F., relative humidity about 65% at atmospheric pressure. The increased charging on increasing the pressure is not noticeable in the case of the brass rubbing plate. The most noticeable feature of the results with a brass rubbing

plate in a humid atmosphere is that the observations are much more irregular than under the other conditions observed. In Curve II a mean curve is drawn through the many points observed but points occur a considerable distance away from it. A pressure run at 69° F. is very similar in form to Curve I, Fig. 8.

### *Direction and Quantity of Charge*

The actual quantity of charge was not measured though the capacity of the insulated system is estimated to be 30 to 35 microfarads. Thus a charge which raised the potential of the electrometer to 100 volts would represent about  $3 \times 10^{-9}$  coulombs of electricity. The charge represents that induced in the electrometer by the charge on the upper surface of the celluloid. The charging was usually of the same sign as that produced when an ebonite rod is rubbed with felt, *i.e.*, negative electricity. In the curves shown the positive voltages represent the usual nature of the charge on the celluloid, *i.e.*, negative electricity. On some occasions when felt was rubbed on the film back the sign of the charge was reversed. Such points are shown as negative voltages (see Fig. 5).

## SUMMARY OF RESULTS

Owing to the nature of these experiments and the material under investigation, the results can lead only to a qualitative analysis of what happens. The qualitative summary is given below and later the application of the results to aerial cameras, in so far as they are applicable, will be discussed.

### 1. *Rubbing Materials*

Of the two rubbing materials tried, a bare brass plate left the celluloid film charged to a potential two or three times higher than that when the rubbing plate was covered with felt.

### 2. *Variation of Charging with Pressure*

(a) *Felt covered rubbing plate.* The variation of charging with pressure reduction followed no definite rule. A steady decrease in pressure from about 76 to about 20 cm. of mercury usually caused small changes (not more than 25 to 40%) in the quantity of charge when the atmosphere was dry (see Figs. 4 and 5). In a humid atmosphere very large changes are likely to occur but these follow no definite rule (see Fig. 8). The most significant feature of pressure variation was the fact that when the pressure is rapidly increased, say, by about 15 cm. of mercury, the quantity of electric charge produced increased considerably but often only the first time the film was rubbed (see Figs. 5 and 8). This phenomenon was particularly noticeable below about 60 cm. of mercury and sometimes was reversed in sense at higher pressures. It also occurred more noticeably at low temperatures than at high.

(b) *Brass as rubbing plate.* The variations in the quantity of charge with pressure are analogous to those in the experiments in which celluloid was rubbed with felt, except that there was a marked decrease instead of an



increase in charging when the pressure was increased (see Fig. 6). Again this appeared only at pressures below about 60 cm. and was more noticeable at low temperatures than at high. In a moist atmosphere this effect seemed either to be absent or to occur in opposite sense (see Fig. 10).

### *Temperature Variation*

(a) *Felt as rubbing plate.* In a dry atmosphere the quantity of charge in general increased with the temperature over the range studied (about 25° to about 65° F.) (see Fig. 7). In a moist atmosphere the quantity of charge did not appear to follow any definite rule with varying temperatures (see Fig. 10).

(b) *Brass as rubbing plate.* In a dry atmosphere the quantity of charge decreased slightly with temperature. In a moist atmosphere it was very erratic and appeared to follow no definite rule (see Fig. 10).

### *Variation with Humidity*

Apart from variations discussed in the two sections above, the most significant fact appearing in the results of these observations was that the quantity of charge generated was greater in a moist atmosphere than in dry, usually by a factor of two or three.

## CONCLUSIONS

The most striking facts derived from the above results are the effects of increasing the pressure and the humidity. Obviously the triboelectric effect of celluloid film is intimately associated with the atmosphere in which it is rubbed. The same probably applies to felt and brass, as the effect of increasing pressure in dry air is opposite in the two cases. The unexpected increase in quantity of charge generated in a moist atmosphere also indicates that the layers of gases and vapors on the surfaces of the materials have a profound influence on the triboelectric effects.

Other investigators (1, 4, 5, 7, 8) also find that the triboelectric effect observed on rubbing various materials such as ebonite, silk, glass, quartz, sulphur and various metals depends to a great extent on the gas surrounding the specimens. The variations found by different authors however cannot be compared because of great differences in experimental method and combinations of materials used.

Before discussing the application of the above results to the reduction of the static markings on films, let us consider as nearly as we can the conditions under which the films are used in practice in Canada. The temperature of the outside air under photographic conditions is usually somewhere between about 15° and 40° F., though it might vary even more. If a cabin plane is used, which is often the case, the film magazines are kept in the cabin which is heated perhaps to a temperature 20 or 30 degrees higher than the outside air. The camera, on the other hand, being partly inside the cabin and partly outside will be at some intermediate temperature below the temperature of the film magazine. Thus when the film is unrolled and passes over the focusing plate it may be subjected to a sudden temperature reduction. According to

the above results, if the humidity were zero a drop in temperature should tend to reduce the tendency to electrostatic charges. However, the humidity will not be zero. There is a certain amount of moisture in the film when it is rolled up and further, if the plane is flying at a level where the relative humidity is high, the humidity in the film magazine would tend to be medium or high. To obtain a significant measure of the humidity near the surface of the film in a camera under operating conditions would be difficult, but it would not be unexpected to find it considerably above 50%. Under these conditions the effect of a temperature drop remains uncertain but might easily enhance charging. The pressure during a photographic flight would not be expected to change very much unless considerable changes in altitude took place, which as far as the author knows is not the usual practice. The slow reduction of the pressure on ascent from ground, combined with a reduction in temperature, might well cause a great enhancement of the charging effect as indicated by Fig. 8.

These observations, of course, are based on experiments with a rubbing plate of either felt or bare brass. In some of the older cameras felt was used but in newer models the pressure plate was covered with an electrically conducting black paint. The effect of rubbing a film with such a painted plate was not studied. A further difference between laboratory experiments and practice was that in practice new film is constantly being brought under the so-called rubbing plate, while in the laboratory the rubbing was done on the same piece of film throughout a series of runs. It is well known that the effect of rubbing changes the electrostatic characteristics of the surface of the film. It was intended that if the results of the above experiments in the tank indicated definitely conditions under which charging did and did not take place, further experiments would be performed requiring more elaborate apparatus and much more time. For instance, fresh film could be used for each point under observation. Further, the front, or sensitive side of the film, also has electrostatic properties which are responsible to some extent for static markings. The results of the experiments that have been done indicate that under practically any conditions the celluloid is liable to acquire a considerable electrostatic charge when rubbed, and that the charging is particularly sensitive to changes in physical conditions of the film and atmosphere.

### III. Possible Methods of Reducing Static Marks on Films

#### 1. *Choice of Atmospheric Conditions*

From the results of the laboratory experiment on rubbing felt and brass on celluloid films it is evident that the atmospheric conditions in which static is least likely would be the dryest and coldest available. The observations on relative humidity of the atmosphere described previously (2) indicate an altitude slightly above the cloud level, at which the relative humidity is a maximum. Such a maximum appears to exist even on cloud-free days. If flying at this level during photographic operations could be avoided a reduc-

tion in static might be found. If there are any scattered clouds in the sky the altitude can be determined easily from the cloud level. The undesirable region is from the level of the base of the clouds up to about 2000 ft. higher.

However, as discussed in the last paragraphs of the previous section, the applicability of these results is somewhat uncertain as the history of the film immediately previous to exposure is important. Keeping the film magazines at the temperature of the camera, that is, not heating the cabin where they are kept, in fact, keeping them as cold as possible might be advisable—though, unless the air in the magazine is dry, low temperatures are of doubtful value.

## *2. Process of Rolling and Loading the Film*

It was suggested that with a view to reducing charges of static electricity the film should be used in as moist a condition as possible without danger of spoiling the sensitivity of the photographic emulsion. The results of the laboratory experiments indicate that the film back is more likely to acquire charges when in a moist atmosphere than in a dry one. However, there are two effects involved, (1) the actual source of the electrical charge, and (2) the dissipation of the said charge without a spark. If the film were very moist the water which adheres to the surface of the celluloid would make it more or less an electrical conductor and hence the charge might be dissipated without leaving the objectional marking on the photograph. Without considerably more experimental work, it is impossible to say what humidity would be required to make the surface conductivity sufficiently great to remove the possibility of charges building up sufficient potential to mark the film. Very high humidities (near 100% relative humidity) would no doubt solve the problem but such humidities would probably also shorten the life of the photographic emulsion unreasonably.

## *3. Conducting Film*

The ideal way to entirely eliminate static markings is to make a film which is an electrical conductor. During a consultation with engineers of the Eastman Kodak Company, the author was informed that they have been trying for years to produce a conducting film, without much success. If it were not necessary to use a transparent film the problem would not be so difficult. For instance, if the emulsion could be put on a thin steel ribbon instead of celluloid, no static whatever would be found. However, the process of making prints would have to be completely altered because of the opaque film. Reflected light would have to be used. No doubt there would be many other difficulties to be overcome, but even if steel were found to be entirely unsuitable, probably some conducting material could be found just as suitable as celluloid except for transparency.

It is understood that the Fairchild Aerial Camera Corporation has carried out experiments in which a sheet of metallic foil is wound on the film spool with the film and runs through the camera back of the film, so that no appreci-

able rubbing takes place on the film back. This should remove static markings due to charges on the film back. No information as to whether sufficient tests have been made to draw any conclusions has been made available.

An alternative suggestion is, that instead of backing the film by a metal sheet a series of fine metal wires be imbedded in the celluloid film so that the charges accumulating on the film would have a much shorter path over the surface of the film before being dissipated by conduction to the camera.

#### 4. *Covering on the Pressure Plate and other Surfaces at which Static Arises*

In Section 1 of this paper some preliminary experiments were described which indicate that probably the best material with which to cover the pressure plate is the emulsion side of a piece of film of the type used in the camera. The Fairchild Aerial Camera Corporation, acting on the results of such experiments brought out static eliminating adaptors for their cameras. The adaptor is a cover for the pressure plate and appears to be a piece of black celluloid with edges molded so it can be slipped over the plate and stay in place. Combined with these were insulated covers for the two aluminium strips which hold the glass focusing plate in place and rollers made of some electrical insulating material, such as bakelite. The object of these was to cover with insulating material the metal parts to which sparks were likely to jump, as described in Part I of this paper.

The Department of National Defence used some of these static eliminators in test flights and in operations in 1931. They very kindly sent a copy of their analyses of static encountered in R C.A.F. Photography, 1931, to the National Research Council. A summary of their analysis is made in Table I, which also includes other appliances supplied by the National Research Laboratories. Totalling all static troubles one finds that with no eliminators 17.6% of the rolls of film were affected. With the Fairchild static eliminators only

TABLE I  
SUMMARY OF RESULTS OF STATIC ELIMINATORS USED IN 1931

Camera equipment	No. of rolls showing effect indicated				Total no. of rolls	Remarks
	Nil	Slight	Medium	Considerable		
Ordinary	909	101 9 2%	51 4 6%	42 3 8%	1103	17.6% affected by static
Fairchild eliminator	75	1 1 2%	1 1 2%	5 6 1%	82	8 5% affected by static
Insulated rollers	2	1			3	
Non-insulated rollers	1				1	
Radium	2				2	

8.5% of rolls used were affected. This indicates about 50% reduction in the total number of rolls affected by static. The reduction was mainly in the rolls which had only slight or medium quantities of static, but under conditions where considerable static (see Column 5) was found there is no reduction but an increase in the percentage of static. The increase may not be significant but it does appear that under bad static conditions the Fairchild eliminators are not successful, though the reduction of the total quantity of static is a step in the right direction and the Fairchild static eliminators on the whole represent an improvement.

##### 5. Alterations in the Design of the Camera

Probably the most effective alteration in camera design would be to raise the pressure plate higher and raise the rollers somewhat as well, so that the film would not touch either the pressure plate or the glass focusing plate. As the film curls towards the focusing plate at the edges probably the clearance between the level of the bottoms of the rollers and the glass plate need not be as great as that between the former and the pressure plate. The pressure plate should be raised as high as possible.

In most of the films having static markings, static rarely appears along the edge of the film but is usually concentrated in the centre, the two inches along the edge being relatively clear. Other devices such as the roller on the cam controlling the mechanism which adjusts the distance the film moves, would probably be better placed near one edge and made as small as possible.

Another alteration which has been tried is in the rollers which guide the film in passing under and out from the pressure plate. Two pairs of rollers were made according to Fig. 11. These were sent to the Department of

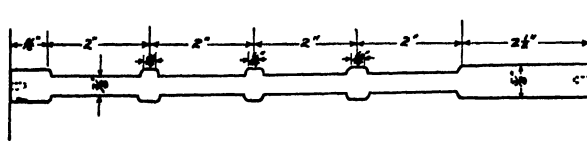


FIG. 11. *Static reducing roller tried in the Fairchild aerial camera.*

National Defence to be tried in film magazines. The rollers were made of aluminium. One pair was coated with a thin layer of celluloid made by dissolving a clean piece of film in amyl acetate and painting several coats of it on the rollers. The other pair was left bare. It was thought that by this means the static markings due to sparks jumping to the rollers might be reduced to the few points where the film touches the roller. This might in some cases save retaking the pictures as a much smaller area of the picture would be spoiled. These rollers have not been given sufficient tests, as only three rolls of film have been used in magazines equipped with the celluloid-coated rollers and one roll in a magazine equipped with the bare aluminium roller. Of these only one roll showed any static and this appeared only in lines where the shoulders on the rollers touched the film. In this case the rollers used were those coated with celluloid.

## 6. *Ionization of the Air in the Camera*

The object of ionizing the air in the camera is to make it electrically conducting so that any charges generated electrostatically will be dissipated without a spark. The difficulty is that any source of ionization in the film magazine is likely to be a source of light which would spoil the film. Radio-active material like radium might be put in the camera at chosen points so that sufficient ionization would be produced without spoiling the film as it moved through the camera. This was tried. A small quantity of radium (about 0.065 mg.) was suitably placed on an aluminium strip which was fastened to the edge of the pressure plate so that it was immediately over the film as it came out from under the plate. Tests were made to see that the quantity of radium was not sufficiently great to mark the film. If the film were left standing still in the camera for 10 min. or more a faint mark on the film appeared at the edge of the pressure plate. This would not spoil a picture. If the film were rolled through the camera as rapidly as is the custom in practice, no appreciable marking appeared.

The magazine equipped with radium was used only on test flights and only two rolls of film were exposed in it, neither of which showed any static markings; hence there is not enough evidence to say anything about its success.

Another method of ionizing the air in the magazine would be to employ the brush discharge or corona discharge which comes off point electrodes charged to a high potential in air. Such equipment is used in printing presses and weaving machinery to prevent trouble due to charges on the paper or threads.

The brush discharge neutralizer has the disadvantage that there is a certain amount of light produced which would spoil the film. It is possible that this light could be shielded from the film and the ionized air circulated to strategic points to discharge the film without a spark. Also a source of high alternating voltage is necessary. This does not, however, offer any great difficulty as a small induction coil would be sufficient. It is understood that the Fairchild Aerial Corporation is considering tests with equipment of this sort.

## 7. *Maintenance of Ground Pressure in the Camera and Humidifying the Magazine*

The fact that static does not seem to be present in cameras used on ground levels leads to the consideration of the maintenance of ground conditions in the camera during operations. This would involve heating and sealing so that the pressure, temperature and humidity would remain fixed. To keep the pressure at that existing at ground level is the most difficult, and in view of the results obtained in the experiments discussed in Part II of this paper it may not be necessary. If the camera is humidified or heated or both, the old problem of lens fogging arises. A method of overcoming this by heating the lens and using the light filter as a sort of double window with the space between supplied with a drying agent would no doubt be developed. The question of humidifying is doubtful in view of the results obtained when felt and brass are rubbed on a piece of film and the charging measured. The

charge appeared to be greater at relative humidities around 50 or 60% than in a dry atmosphere. However as has been discussed previously, if high humidities were used, the increased surface conductivity might prevent the accumulation of sufficient charges to damage the film.

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# AN INVESTIGATION OF THE DENSITY OF A VAPOR IN EQUILIBRIUM WITH A LIQUID NEAR THE CRITICAL TEMPERATURE<sup>1</sup>

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## Abstract

An account is given of the outstanding work done and theories developed in connection with the critical phenomena during the last century. It was because of certain observations made in this laboratory a short time ago upon reaction rates at the critical temperature that the present investigation was begun with a view to providing more definite data concerning the density of both the liquid and gas in the region of the critical point.

Experimental conditions have been kept under the most rigid supervision and previous errors eliminated or evaluated. A distinctly new technique has been developed, utilizing quartz spirals, for determining the density of both the liquid and gaseous phase almost simultaneously, up to, and past, the point where the meniscus vanishes. The results have been compiled from a great many observations taken over a period of two years upon eight separate units. In general, good agreement has been obtained in all but one case, and a probable solution has been advanced for the exception. Primarily, the paper is an experimental one designed to fill an important gap in previously recorded data.

Extensive theoretical deductions have been purposely omitted because of the radical nature of the findings; and it would be necessary to proceed further with the work before anything really definite might be concluded. Briefly, it might be stated that the results cast considerable doubt upon Van der Waals' classical theory of the continuity of state.

## Introduction

The work of Sutherland and Maass (18) in this laboratory upon reaction rates under critical conditions provided evidence of the discontinuity of state. With a view to investigating this condition from another angle, a series of observations and measurements has been undertaken to determine if this discontinuity manifests itself in a number of different physical aspects. The work described here deals with density measurements upon the liquid and gaseous states by a direct method which has several points of superiority over any previous attempts.

The phenomena accompanying the critical temperature have been under observation at one time and another for the last century. Numerous explanations have been offered to account for the observed phenomena, and many determinations upon the existing densities have been tried. A cursory account of the results and their methods of determination will serve to show to what stage the work had progressed when the present investigation was begun.

Almost immediately after Cagnard de La Tour (9, 10, 11) had first observed the disappearance of the meniscus at the critical temperature, two explanations for the phenomenon were advanced. (i) The surface tension of a liquid decreases with rise of temperature and if this diminution continued it would be possible to arrive finally at a temperature at which the surface tension

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had fallen to zero. There would be no capillarity and no surface of demarcation existent; in other words the liquid and gas would be visibly indistinguishable and mutually miscible in all proportions. Whether this means that they are physically identical or not, does not follow directly. (ii) As the temperature rises, the vapor density increases and the liquid density decreases; thus it is conceivable for the two to have finally the same density. Ramsay (14, 15) and later Jamin (7, 8) postulated this latter theory to account for the critical phenomenon.

Cailletet and Colardeau (2, 3) seemed to believe that the liquid and gaseous states persisted separately after the critical point had been exceeded. To substantiate this theory, Cailletet and Hautfeuille (4) experimented with iodine and carbon dioxide in a tube. Above the critical temperature, that portion of the tube previously occupied by the liquid retained its violet color while the upper part of the tube remained colorless. This provided fairly substantial qualitative proof of the hypothesis.

The density determinations made by Cailletet and later by S. Young (24) were performed in such a manner as to require observations upon the position of the meniscus. Of necessity the readings had to be discontinued a short distance below the critical temperature. A similar objection may be raised in connection with all the density values obtained in this manner. In spite of this fact, all the investigators extrapolated the results to the critical temperature in the form of a smoothly curving parabola. The general shape of these curves has been similar in all cases, but the exact nature of this parabola in the region of its apex has in no instance been determined experimentally. Its shape has been inferred but never proved. Admittedly, measurements have been made very close to this region, but very close seems hardly good enough when it is considered that densities are known to have been changing at the rate of 4 or 5% per  $0.1^{\circ}\text{C}$ . This shortcoming has been realized to be of considerable significance and a serious handicap in the physical explanation of the phenomena.

The work of Galitzine (5) followed much the same procedure and yielded the same general results as the preliminary work carried on by the writers. Galitzine found that when using ethyl ether, the temperature at which the meniscus disappeared,  $T_c$ , was not the same as the temperature at which it reappeared  $T'_c$ , and that  $T_c - T'_c$  had a positive and constant value, uninfluenced by the relative mass of the material under observation. Furthermore the position in the tube which the meniscus took up for any temperature below the critical temperature was governed by whether that temperature was approached from above or below. Galitzine seemed convinced from the observations that the density in the upper part of the tube containing the ether was considerably less than the density in the lower part, for even 6 or  $7^{\circ}\text{C}$ . after complete homogeneity was apparent to the eye. He stated that this density difference was about 20% and that it maintained itself indefinitely. Small traces of impurities, notably air, were thought to be responsible, but experimental tubes with definitely greater quantities of air included showed no difference from the results obtained in the tubes carefully filled to exclude air.

Several years later, Traube (21) and Teichner (20) attacked the same problem in a slightly different way than had any of the previous workers. Since all the earlier density determinations depended for their evaluation upon the position of the meniscus, and since the meniscus became indistinct and disappeared just at the stage where the measurements were of the greatest significance, it was logical to determine the density in some other manner. The simplest and most direct method possible was employed. Small glass floats, 1 to 2 mm. in diameter of known density were enclosed within the experimental tube along with the material under observation. The effective density of each float was determined, and each float was individually distinguishable, so that at a glance it could be identified with its correct density value. Traube enclosed eight of these small glass floats in each tube; the heaviest had a density of 0.678 and the lightest, about 0.422; the intermediate ones varied from one another by about 0.035. The liquid-vapor system under investigation was carbon tetrachloride.

Teichner increased the number of floats to 15, thereby decreasing the gap between floats by one-half. Both Teichner and Traube took precautions to ensure a steady temperature capable of precise adjustment, but whether they were successful in obtaining a constant and equal temperature throughout the length of the bomb (16 to 20 cm.) has not been recorded, nor did they seem to have any device to detect such an equality had it been present. Both found a density difference of considerable magnitude between the upper and lower parts of the tube after the critical temperature had been exceeded.

The obvious difficulty in connection with the previous method was the gaps between the density values for each of the floats. The number of floats could not have been increased to any greater extent because of the limited space and the difficulty of identification.

F. B. Young (23) after a consideration of the results of previous workers decided that the marked differences in density were to be attributed to the presence of a small percentage of impurity in the substance assumed to be pure. This would serve to reopen the controversy between Traube's liquid-genic and Andrew's classical theories, and to make additional experimental data relating to the critical phenomena of pure substances highly desirable. Young's procedure for filling the experimental tubes with pure substance was exceedingly precise and has been treated at great length in his account of the work. The actual density figures at the critical temperature were obtained in practically the same manner as was employed by Cailletet and were subject to the same criticisms. Young also used a number of close parallel lines set up behind the tube and observed them through the contents of the tube. When the lines showed no discontinuity throughout their length it was assumed that the density along the entire length of the tube was uniform. Furthermore the behavior and distribution of the opalescence were construed to prove certain facts about the distribution of the densities. Gaseous impurities were credited with retarding rather than assisting the formation of opalescence and the rapidity with which the opalescence spread

throughout the tube was hence a measure of the purity. Young believed that with the disappearance of the fog there vanished simultaneously all density differences and since impurities delayed the spread of the fog as well as shortened its duration, he concluded that gaseous residuals were entirely responsible for the differences in density reported by previous workers. It might be well to note that Young considered density equilibrium to have become established throughout the tube during the lapse of a few minutes (he mentions 10 min. in one place) following an alteration in temperature. He ascribed even this lag to the time required for thermal conduction through the walls of the containing vessel.

P. Hein (6) conducted his investigation of the subject in an analogous manner to Traube and Teichner. His results were not in exact accord with theirs. He employed a thermocouple in the heating bath but he did not state how accurate it was nor to what extent it was used. In general, he found that the density differences might be attributed to gaseous impurities but that rapid stirring, performed magnetically within the bomb, offset the effect of the impurities and removed the density difference. In other words he implied that the gaseous impurities only retarded equilibrium and because of this retardation a density difference had been obtained. This conclusion would be valid only if no temperature gradient had existed throughout the length of the bomb, a point which he does not make clear.

Schröer (16, 17) obtained a density-temperature curve with its apex decidedly flattened, but it is to be feared that his pressure manipulation was entirely to blame for this result. Many other investigators have explored this region but have contributed very little reliable information subject to experimental verification.

Even a hasty examination of this short review is sufficient to show that the results, obtained over a considerable number of years, have been at variance amongst themselves. In general, two main divisions seem to have developed; (i) those who have clung to Andrew's classical theory and endeavored to explain the phenomena by reference to his pressure-volume isothermals, and (ii) those who have believed, like Traube, that an intermediate formation of liquidons and gasons must be necessary.

There have been a number of entirely independent investigations involving critical temperature-pressure regions which point to a discontinuity of state at the critical temperature. It has been observed that the solubility of a substance decreases markedly as the critical pressure is approached and reaches a zero value in its neighborhood, which cannot be accounted for by a change in concentration of the solvent (1). Kamerlingh Onnes (13) has determined the dielectric constant of a system through the critical temperature-pressure region. A discontinuity which he has not interpreted seems to be obvious from his data.

The above references are given as evidence that in spite of the apparent continuity of the pressure-volume isothermals a definite discontinuity must exist at the critical temperature. The solubility, velocity of reaction and

dielectric discontinuities might be explained by a regional orientation which may exist in the liquid state, the disappearance of which may greatly influence the so called critical temperature. The suddenness of the disappearance of such a regional orientation with slight temperature changes has already been proved by a dissertation of Nernst's (12).

Apart from this, the existence of a liquid and vapor density difference persisting beyond the critical temperature becomes more or less obvious from the observation that the critical temperature (disappearance of the meniscus) can be observed in tubes containing various amounts of material under observation. On the basis of Van der Waals' theory of the continuity of state, the following calculation will show that the critical phenomenon would be observable only when the tube was filled with the material under inspection to an extent which will conform to a *single* definite critical density value.

Suppose  $W$  = weight of material;  $V$  = volume of tube;  $V_g$  = volume of gas, and  $d_g$  = density of gas;  $V_l$  = volume of liquid, and  $d_l$  = density of liquid;  $d_c$  = density of contents at the critical temperature.

Under all conditions:

$$(V - V_g)d_l + V_g d_g = W,$$

$$\text{Hence } V_g = \frac{W - Vd_l}{d_g - d_l}.$$

As  $T$  approaches  $T_c$ , then  $d_c$  approaches  $\frac{d_g + d_l}{2}$

And  $W \doteq d_c V$ .

Substituting these special conditions in the general equation,

$$V_g = \frac{d_c V - d_l V}{d_g - d_l} = \frac{\left[ \left( \frac{d_g + d_l}{2} \right) V \right] - Vd_l}{d_g - d_l} = \frac{V}{2} \left[ \frac{d_g - d_l}{d_g - d_l} \right]$$

$$\text{Hence } V_g = \frac{V}{2}$$

It is seen that the writers are inclined to question the continuity of state in the absolute sense in which Van der Waals advanced it. The discrepancies indicated by the solubility and velocity of reaction experiments cannot be controverted. The discrepancy existing between liquid and vapor densities, implied by the above calculation, and indicated by the experiments of Traube and Teichner, cannot be made to conform to the continuity theory except on the basis of experimental influences involving a vertical temperature gradient, the presence of an impurity, or a pressure gradient based on gravitational attraction. From the point of view of the inflection in the pressure-volume isotherms which gives  $dP/dV=0$ , it is admitted that the slightest

variation in experimental conditions would tend to bring about an observed variation in density. One of the objects of the work described herein was to eliminate such variations as might be caused by a temperature gradient, or contamination by an impurity; at least to evaluate their influence where a complete removal was prevented by experimental exigencies. The first step in this direction was the adoption of a technique for determining the absolute densities in both the liquid and vapor phase independently under conditions where true equilibrium existed.

### **Preliminary Experimental Work**

The methyl ether was enclosed in a U-shaped tube at first, and was observed at the critical temperature with the arms of the U uppermost, then the tube was inverted and the methyl ether observed again. Observations were then made on a fresh sample of ether enclosed in a straight tube. The usual phenomena were noted, but by making cathetometer readings of the position of the meniscus at various temperatures while approaching the critical temperature and again while receding from the critical temperature, a peculiar fact was brought out. The height of the meniscus above the bottom of the bomb in the first instance was not the same as the height from the bottom in the second case, both at the same temperature. More peculiar still, the difference in the two readings was constant for five observations taken within  $2^{\circ}$  C. of the critical temperature. It might be well to note here that the bombs were about 30 cm. in length and had an internal diameter of 1.4 cm. It was hoped that this container would reduce the equilibrium lag to a minimum.

Following this last observation, a new bomb was constructed of approximately the same dimensions but having included in it a glass float about 3 cm. long and 0.5 cm. in diameter. The density of the float was made as close to that of the ether at the critical temperature as was possible. The obvious difficulty was the terrific pressure which such a float had to withstand. Many attempts were made before one was obtained, and the successful one had a few drops of the liquid ether included within the float to partly compensate for the external pressure to which it was subjected. Even then it sank through the liquid at a temperature about  $4^{\circ}$  C. below the critical. A little consideration will show the impossibility of constructing such a float with an effective density equal to, or less than, the density of the vapor at the critical temperature.

The only useful information derived from the preceding experiments was the fact that the bomb tubing employed could successfully withstand the pressures and that the float might be useful if some modifications were introduced. It was at this point that the idea was conceived of continuing the measurements with the floats, but suspending them from a quartz spiral whose extension would give a means of calculating the fraction of the weight of the float not being held up by the buoyant effect of the surrounding medium. It was also thought necessary to have some means of moving the device to various parts of the interior of the bomb. The technical difficulties involved

in such a scheme seemed at first to preclude its realization. The space available was limited by the structural strength of the glass bomb since its internal diameter could not be increased and still continue to give any margin of safety against explosion. Furthermore the moving device had to be compact and such that it would not interfere with the free movement of the float when readings were to be taken and yet allow of complete control from the outside.

### Description of Apparatus

The quartz spirals employed were prepared by a machine designed and constructed in this laboratory (19). The spirals had a sensitivity comparable in every respect to that of an analytical balance and yet occupied a space not in excess of 4 cm. in length and 0.5 cm. in diameter.

The bomb (Fig. 1, *A*) was constructed from a section of thick-walled Pyrex tubing 18 in. long, closed at one end. A slight depression was made in the wall at a point 14 in. from the closed end (just below *B*). A piece of thin-walled Pyrex tubing was then selected which just fitted snugly within the bomb tubing but which came to rest against the constriction at *B*. A piece of this tubing  $\frac{3}{8}$  in. in length, was fitted with a glass pulley, made from capillary tubing, which revolved freely upon a glass shaft fastened at both ends to the upper edge of the ring. Upon the lower edge of the ring were two glass guides, one at the centre and the other at the edge.

A thin wire nail was fitted loosely into a Pyrex sheath with very thin walls, and the sheath allowed to extend a short distance past both ends of the nail (*E*). This particular design prevented the nail from sloping away from the interior wall of the bomb at the end opposite to which the magnet was applied. A small glass hook on one end completed this unit.

The counterweight (*W*) was a piece of capillary tubing adjusted in size so that its weight was almost equal to that of (*E*). A closed hook at the top and an open hook at the bottom completed this part.

The float (*D*) was of Pyrex glass; its shape was uniform and of such a design as to best resist high pressures without collapsing. The volume was made as near to 1 cc. as possible and its weight kept between 0.4 and 0.5 gm. A neat open hook was sealed to the top of it. The accurate determination of the volume of the float presented considerable difficulty. A small specific gravity bottle was constructed with a wide mouth closed by a ground glass stopper and a capillary overflow from the opposite end. The float itself was weighed

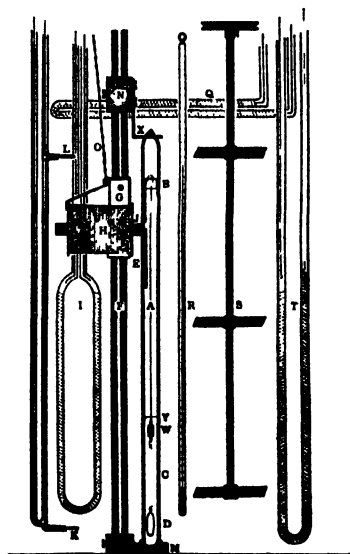


FIG. 1. Diagram of apparatus for heating the experimental tubes through the critical temperature, showing the construction of the moving spiral and float device and the general lay-out of the various parts.

and the weight corrected for the buoyant effect of the atmosphere. The specific gravity bottle was weighed full of distilled water at a known temperature, and again full of water with the float included within it. In this way a consistent determination of the volume of the float could be obtained.

A fine silk thread was tied to the glass-sheathed nail (*E*), then threaded up through the outside guide on (*B*), thence over the pulley and down through the centre guide to the counterweight (*W*). The length of the thread was such that with the nail about 1 in. from the bottom of the bomb, the counterweight (*W*) was against the saddle (*B*). The open hook of the float (*D*) was slipped into the ring at the bottom of the calibrated spiral (*C*) and the hook carefully closed using a small flame. Similarly the hook on the bottom of the counterweight (*W*) was connected to the top ring of the spiral. The whole suspension was lowered into the bomb.

The bomb was clamped in an upright position and immersed in water up to the level of (*B*). At a point 2 in. above (*B*) a uniform constriction was made in the tube, leaving only a narrow aperture. This was done because the silk thread had to be kept cool while it was only about 1 in. removed from molten Pyrex. Furthermore it was desired to leave as little "dead-space" as possible above the saddle. A short piece of Pyrex tubing was then sealed on to the open end of the bomb and connected to the filling device. (See Fig 2, *T*.)

#### *The Methyl Ether Preparation and Purification Train*

With reference to Fig. 2, the apparatus may be divided roughly into three sections, the divisions being based upon the use of each section.

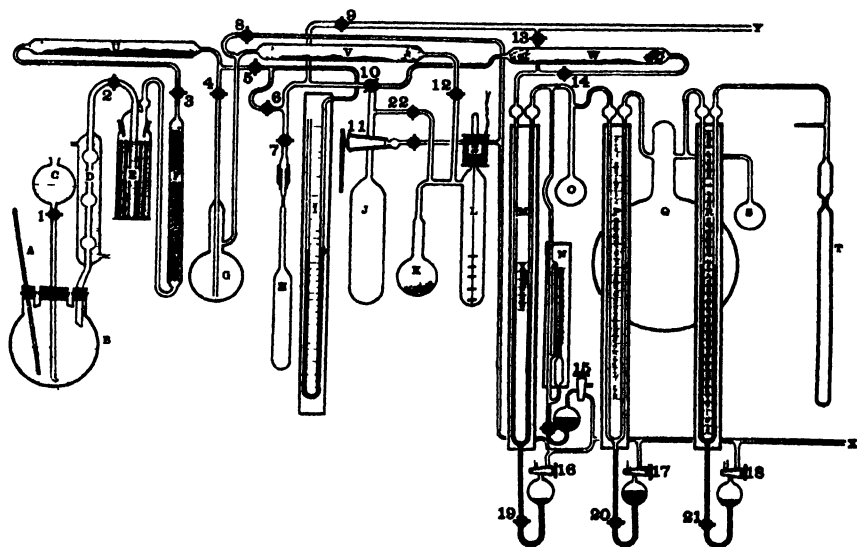


FIG. 2. Diagram of apparatus for preparing, purifying and delivering pure methyl ether to the experimental tubes.

(i) (Beginning at the left.) From (*A*) to (*G*) was designed for the preparation and crude purification of the ether.

(ii) From (*H*) to (*L*) was used for the further refinement and delivery to storage bombs, as well as for introducing the ether back into the system from storage when desired.

(iii) The section (*M*) to (*S*) served to admit measurable quantities of pure ether to the finished bomb without the danger of contamination with air or stopcock grease.

(*B*) was a one-litre Pyrex flask fitted with a 0° to 360° C. thermometer (*A*), a dropping funnel (*C*) and a water-cooled condenser (*D*). (*E*) was a bubbler packed with glass rods and filled with concentrated sulphuric acid. Tubes (*F*) and (*U*) were supplied with calcium chloride lumps and phosphorus pentoxide, respectively. (*G*) was a 300-cc. Pyrex flask.

In operation, 500 cc. of concentrated sulphuric acid was added to (*B*), stopcocks Nos. 2, 3 and 4 were opened and a refrigerant (−80° C.) was placed around (*G*). Stopcock No. 5 was turned so as to connect the system to the mercury manometer (*I*). A bunsen burner was placed under (*B*) and stopcock No. 8, which communicated with the water vacuum line, was manipulated so as to maintain a negative pressure of about 4 cm. of mercury as registered on (*I*). When the thermometer (*A*) reached 130° C., the methyl alcohol in (*C*) was admitted drop by drop to (*B*) through the glass valve No. 1. The temperature was kept steady and the pressure maintained as described. Liquid methyl ether collected in (*G*).

Stopcocks Nos. 2, 3 and 4 were closed and No. 12 opened, the refrigerant was transferred from around (*G*) to (*L*) and the methyl ether slowly distilled from (*G*) to (*L*) passing through the phosphorus pentoxide drying tube (*V*). The container (*L*) had about one-half the capacity of (*G*) and was fitted with an electromagnetically operated glass stirrer. When (*L*) was three-quarters full, stopcock No. 12 was closed, the refrigerant was replaced about (*G*) and also around (*K*). An empty Dewar flask was set around (*L*) and the stirrer started. The ether very slowly distilled, without bumping, into (*K*). The flask (*K*) contained about 4 gm. of sodium wire, which it was hoped would take out the last traces of water and alcohol. The last portion (15 cc.) in (*L*) was allowed to escape each time. From (*K*) the ether was distilled into (*J*). The remainder of the ether which had been left in (*G*) was put through (*L*) and (*K*) as previously described and finally added to that already in (*J*).

The storage bomb (*H*) was made of ordinary glass tubing and could have any desired capacity from 10 to 50 cc. It was fitted inside the glass tubing just below No. 7, and the joint made air-tight by a section of rubber tubing wired in place. Stopcocks Nos. 6 and 7 were opened and No. 11 was closed; No. 10, a two-way valve, was turned so as to connect (*J*) with (*H*). Vacuum was applied by opening No. 9, which communicated with (*Y*) the high vacuum line. When (*I*) registered a good vacuum, No. 9 was closed and the contents of (*J*) allowed to warm up to about 0° C. This served to create a pressure of about two atmospheres in (*H*). A momentary opening of No. 11 returned



the pressure in (*H*) to atmospheric, which was again reduced, as before, to a vacuum. After about three repetitions of alternate vacuum and pressure, the refrigerant was placed around (*H*) and stopcock No. 11 carefully adjusted so as to maintain a pressure of about one atmosphere as read on the manometer (*I*). Ether (50 cc.) could be transferred in this way from (*J*) to (*H*) in about 4 min.

When (*H*) was *four-fifths full* of liquid, valve No. 11 was closed and the stopcock No. 9 opened, the cooling mixture was left around (*H*) and a flame applied to the constriction in the neck of the bomb. When the constriction collapsed the bomb was removed and could be kept until needed at room temperature. Other bombs were placed in position and filled as before until the supply in (*J*) became exhausted.

The section of the apparatus on the right of the diagram consisted essentially of three mercury seals (*M*), (*P*) and (*R*). On (*P*) and (*R*) were carefully calibrated scales extending their full length, while (*M*) had only a short section of rough scale at its central point. Each branch of the U in every seal was topped by a 50-cc. bulb in order to prevent mercury from being forced through the system when one or other of the seals was pulled out while a difference of pressure existed on opposite sides of it. Even with this precaution, under pressure differences of more than 10 cm. of mercury, the seal could not be successfully withdrawn. Stopcocks Nos. 16, 17 and 18 were of the two-way variety, one lead communicated with the water vacuum line (*X*), while the other opened to the atmosphere. Stopcocks Nos. 19, 20 and 21 were ordinary one-way valves which were necessary to arrest the upward or downward motion of the mercury at the will of the operator. The length of the arms of each mercury seal was roughly 120 cm.; the height from the mercury level in the reservoir to a point half way up the U was 76 cm. The mercury was carefully cleaned and dried and its level was never allowed to fall below any one of stopcocks Nos. 19, 20 and 21.

(*N*) was a McLeod gauge capable of measuring pressures down to 0.0002 mm. of mercury. (*O*) was a 50-cc. Pyrex flask, (*Q*) was a Pyrex round-bottom flask of roughly six-litre capacity. It was planned at first to use this calibrated volume for introducing accurate quantities of gaseous methyl ether to the bomb, but in practice it was never used as such. The small bulb (*S*) was introduced to enable the gaseous methyl ether which had been accumulated in (*Q*) to be condensed, thus allowing the mercury seal (*R*) to be pulled out without forcing mercury out of (*R*) into (*T*).

All the mercury seals were drawn out, stopcock No. 14 was closed and No. 13 opened thereby connecting the system from (*M*) to (*T*) with a Langmuir mercury pump backed up by a mechanical high vacuum pump. The evacuation was allowed to continue for a period of 4 or 5 hr., at the end of that time the pressure had been reduced to about 0.0003 mm. of mercury. The seal (*M*) was raised to the position shown in the diagram and the extent of the vacuum was tested occasionally over a period of 2 hr. to detect, if possible, any leaks. Very little trouble was encountered in making the

system air-tight, and on several occasions the pressure did not appreciably alter even when left for 24 hr.

When testing the system for leaks one of the methyl ether storage bombs of 35 or 40 cc. capacity was placed in a refrigerant ( $-20^{\circ}\text{C}.$ ) for 5 min., then removed, wrapped in a towel, and the tip broken off. The open end was then inserted below stopcock No. 7 and held there in the manner described previously. The contents was allowed to distil over into (*J*) and held in readiness. The phosphorus pentoxide drying tube (*W*) was replaced by a fresh one every time a new bomb was to be filled. When the system to the right of (*M*) had been found capable of maintaining a vacuum, stopcock No. 10 was turned so as to connect (*J*) with (*W*), stopcocks Nos. 13 and 14 were opened and the small volume from No. 11 over to (*M*) was evacuated thoroughly. Stopcock No. 13 was then closed and No. 11 opened until the pressure as indicated on (*M*) was about one atmosphere. The evacuation was then repeated, followed by the building up of the pressure as described. This process was performed about six times to be sure that whatever pressure did remain was due to methyl ether and not to air.

Mercury seals (*P*) and (*R*) were raised to their midpoint and (*M*) was drawn down, a refrigerant ( $-80^{\circ}\text{C}.$ ) was placed around (*O*). When about 25 cc. of liquid had collected in (*O*), the distillation was stopped and about 5 cc. allowed to evaporate out of (*O*) into the pump system (*Y*). The seal (*M*) was immediately returned to its position as shown in the diagram. All the precautions described were taken to preclude the possibility of introducing air into the carefully evacuated system.

The mercury seal (*P*) was drawn out, the refrigerant around (*O*) was removed, and the pressure in (*Q*) was allowed to build up to about 80 cm. of mercury, as registered on (*R*). The mercury in (*P*) was then forced up into position by pressure applied to the atmosphere lead of stopcock No. 17. Simultaneously the refrigerant was returned to (*O*). Another Dewar flask containing refrigerant ( $-80^{\circ}\text{C}.$ ) was placed around (*S*) to reduce the pressure in (*Q*) to such a value that the seal (*R*) could be withdrawn. The refrigerant was then placed around (*T*) and the seal (*R*) immediately drawn down. When the desired amount of ether had been condensed in (*T*) the seal (*R*) was again raised. By means of the oxy-gas flame, the bomb (*T*) was sealed off at the constriction and removed from the system. Incidentally, it was discovered that a bomb filled with liquid at  $-80^{\circ}\text{C}.$  to *twenty-four eighty-fifths* of its total volume gave an experimental tube in which the meniscus disappeared at the critical temperature at a position practically the same as it occupied at room temperature. This generalization has been tried for methyl ether and for ethylene and found to be true in both cases.

### *The Heating Bath*

The accompanying diagram (Fig. 1) represents the arrangement of the heating apparatus with the bomb in position. The actual container (not shown in diagram) was a Pyrex jar, 18 in. high and 8 in. in diameter. The jar contained 3 gal. of glycoline which served as the heating medium. A certain

amount of detail has been omitted from the drawing so as not to confuse with the really essential features; furthermore some of the parts have been slightly misplaced in order that all may be shown more clearly.

The thick-walled Pyrex glass bomb (*A*) contained the methyl ether with the meniscus level at (*Y*). It was securely set in a pocket in the wooden base (*M*) and held at the top by the heavy copper strip (*X*) which had a hole drilled in it large enough to fit over the tip and rest firmly on the shoulder of the bomb. This copper strip was in turn bolted to the collar (*N*) which was set-screwed to the heavy brass rod (*F*). This particular arrangement allowed for the easy removal of the bomb and yet assured that when in position it would be held rigidly in place. Great care was taken to be sure that the bomb was absolutely vertical and that nothing altered this in the process of heating.

The brass shaft (*F*),  $\frac{3}{4}$  in. in diameter, had a key-seat cut along its entire length; the brass collar (*G*) was fitted with a key which allowed the free vertical movement of (*G*) but restricted its rotation upon the shaft to almost zero. Attached to the collar (*G*) was an electromagnet (*H*) wound upon a hollow brass spool with brass ends. Through the brass spool was inserted a solid wrought iron core (*J*) which fitted closely but yet had free longitudinal movement within the spool. The correct length of the core was such, that with the bomb in position, about three-eighths of an inch more of the core projected from the spool at the end remote from the bomb than from the end next to the bomb. The purpose of this was to ensure that the pole piece was kept in direct contact with the exterior of the bomb throughout the latter's entire length by means of the natural spring created by the core endeavoring to centre itself in the electromagnetic field. This may seem like a superfluous refinement, but until its adoption all sorts of difficulties were encountered in getting a firm grip upon the nail.

The chain (*O*) was fastened to (*G*) and proceeded upward and over a suitable sprocket mounted upon a shaft, and thence to a counterweight outside the bath. The shaft had a pulley over which ran a belt that extended back to another pulley and crank situated beside the operator. By turning the crank one way or the other, the magnet device could be raised or lowered at the will of the experimenter, and when the electromagnet was turned on, the nail within the bomb followed along, thereby placing the float (*D*) at any desired position between the upper and lower extremities of its movement.

The heaters (*I*) and (*T*) were connected in parallel with each other and in series with a suitable rheostat which could be adjusted to vary the current from one to nine amperes. Since the temperature control was entirely manual, a switch was connected *across* the rheostat leads, and when the temperature was observed to be falling slightly, this switch was closed for four or five seconds, thus raising the current flowing through (*I*) and (*T*) to a maximum for that length of time. When the switch was reopened the original flow of current was resumed. This dispensed with the difficulty of resetting the rheostat at the correct position after each restoration of temperature. With

a little practice, the temperature control became a comparatively easy matter and needed to be checked not oftener than once every 5 or 10 min. This amount of attention served to maintain the temperature to within one-twentieth of a degree of the desired value at all times.

The heater ( $Q$ ) was on a separate switch and had its own controlling rheostat. ( $L$ ) and ( $K$ ) were the upper and lower junctions of an alumel-chromel thermocouple. Actually ( $L$ ) and ( $K$ ) were exactly duplicated by a second set of similar junctions connected in series with those shown, the purpose being to give greater sensitivity. The carefully insulated leads from the thermocouple were connected to a lamp and scale galvanometer. This device readily detected differences in temperature between the upper and lower junctions of the order of  $0.01^{\circ}\text{C}$ . Using this as a detector, the current necessary to maintain any desired temperature was correctly proportioned between ( $Q$ ) and the double unit ( $I$ ) and ( $T$ ) so that the galvanometer registered no deviation. Taking no precautions, *i.e.*, without operating ( $Q$ ), the top of the bath would become from one- to two-tenths of a degree cooler than the bottom. The position of the junction ( $K$ ) was not as close to the heater ( $I$ ) as the drawing would indicate. Actually, both ( $K$ ) and ( $L$ ) were situated very close to the top and bottom of the bomb respectively, while heaters ( $I$ ) and ( $T$ ) were removed as far as possible from each other and from the bomb. A fourth heater (not shown) was also in the bath, but was used only in conjunction with ( $I$ ) and ( $T$ ) to raise the temperature fairly rapidly in the early stages of the heating, but was never used to maintain a temperature.

The thermometer ( $R$ ) had a temperature range of  $0$  to  $200^{\circ}\text{C}$ ., graduated in fifths, and was calibrated against a standard thermometer over the range of  $100$  to  $150^{\circ}\text{C}$ . The stirrer ( $S$ ) occupied a central position in the bath, was of a sturdy construction involving double bearings top and bottom, and was driven by a belt from a variable speed motor. The stirrer blades were about 3 in. long from tip to tip and were rotated at a brisk rate of speed to eliminate as far as possible any stagnant oil. Directly behind the bomb, but outside the glass containing vessel was a vertical row of four 100-watt frosted Mazda bulbs which provided a good clear illumination throughout the length of the bomb.

The whole bath was thermally insulated with asbestos and rested upon a 50-lb. slab of slate. The entire unit was housed within a sheet-steel tank,  $2\frac{1}{2}$  ft. high, 2 ft. wide and  $1\frac{1}{2}$  ft. from front to back. The front face of the tank was inset with a plate-glass window, 23 in. high and 7 in. wide. Outside of this window were two wooden wings, securely fastened in such a manner as to leave only a sufficiently wide slit to make the bomb and thermometer visible. Ten feet back from this was a tough fibre-board screen, 7 ft. high and 7 ft. across, firmly fastened in an upright position. A plate glass window 2 ft. long and 6 in. wide was set in this screen at approximately the same level as the window in the tank. Behind the screen were all the electrical controls; the cathetometer, for measuring the spiral extension; the telescope for observing the thermometer; and the operator. Both the cathetometer

and the telescope were especially constructed to give a high magnification at the distance for which they were used.

The apparatus as herein described has given good satisfaction for about 500 hr. of actual operation. The factor of reliability was of great importance because the operator could not safely approach the heater to carry out any adjustments, alterations or repairs while the temperature was up.

### Experimental Procedure

In the main, three types of determinations were attempted on each bomb.

(i) The density was measured from the upper to the lower limit of movement of the suspension while the bomb was maintained at some definite temperature.

(ii) The density above and below the position at which the meniscus disappeared was determined at a succession of different temperatures; these temperatures first being approached from below and then from above.

(iii) The effect of a vertical temperature gradient was thoroughly investigated. The importance of removing this gradient will be shown in some detail in the following results.

Since all the weight measurements depended upon the length of the spiral which was measured by optical means, it was necessary to check up on any possible optical distortion brought about by the various layers of glass and oil between the spiral and the eye. No correction was found to be necessary.

In all the bombs, the following data had to be compiled before the parts were assembled:

(i) The volume of the glass float ( $V$ ); (ii) the weight of the float ( $W$ ); (iii) the sensitivity of the spiral ( $S$ ); (iv) the normal length of the spiral ( $N$ ).

With regard to the value of ( $V$ ), it might possibly be thought that under the influence of the pressure encountered under working conditions a slight contraction might be caused, however, up to pressures of 55 atm. no such contraction was measurable; at least if it did exist it was less than the experimental error (22).

With the foregoing information it was possible to determine the density of the medium surrounding the float at any time. The length of the spiral from tip to tip was measured by the cathetometer, say ( $X$ ) mm. From this was subtracted the normal length, ( $N$ ), and the result multiplied by the sensitivity of the spiral ( $S$ ). The product was subtracted from the weight of the float ( $W$ ), and divided by the volume of the float ( $V$ ), the result of this division gave the density. In abbreviated form it may be expressed as:

$$d = \frac{W - (X - N)S}{V} .$$

The cathetometer readings could be checked repeatedly to 0.05 mm., but since two readings had to be made for each density determination, the extreme limit of error might be considered as 0.10 mm. An alteration of this extent in the cathetometer reading would change the density value in a typical case by 0.0005.

## Experimental Results

The following tables have been selected from some 500 pages of data with no regard for chronological sequence. A total of seven different bombs containing varying quantities of liquid have been investigated successfully over a period of some 500 hr. of observation. Obviously, only sufficient figures can be given to indicate the trend of the results.

TABLE I  
RESULTS OBTAINED WITH BOMB NO. 9

I			II			III		IV	
Temp., ° C.	Density (a)	Density (b)	Temp., ° C.	Density (a)	Density (b)	Temp., ° C.	Density (a)	Temp., ° C.	Density (a)
126.3	.2128	.2122	128.9	.2662	.2635	125.9	.3406	128.9	.2722
126.5	.2170		127.3	.2634		126.3	.3332	126.9	.2722
126.7	.2202	.2192	126.9	.2613	.2620	126.7	.3207	126.5	.3020
126.9	.2255		126.7	.2606		127.1	.3138	126.3	.3168
127.1	.2296	.2296	126.3	.2384	.2345	127.9	.2967	126.1	.3288
127.3	.2338		125.9	.2220	.2190	128.9	.2854	125.9	.3367
127.9	.2432	.2449	124.9	.2008	.1977	131.1	.2772		
128.9	.2523	.2535				132.3	.2742		
129.9	.2583					135.1	.2722		
131.1	.2618	.2606							
132.1	.2638	.2624							
135.0	.2662	.2648							

NOTE: See Fig. 3. I, float above visible meniscus, temperature approached from below; II, float above visible meniscus, temperature approached from above; III, float well below visible meniscus, temperature approached from below; IV, float well below visible meniscus, temperature approached from above.

The temperature was equalized throughout the bath. Density (a) and Density (b) represent values obtained on different days.

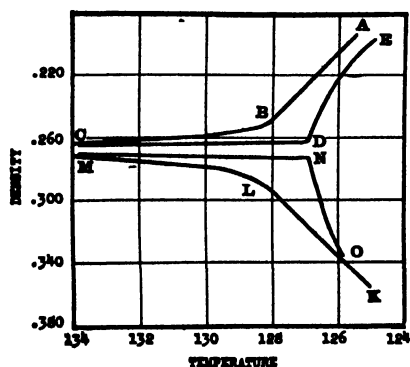


FIG. 3. Shows the relation between density and temperature and the influence of the direction of approach to any particular temperature. Curve ABC corresponds to section I, curve EDC to section II, curve KLM to section III, and curve QNM to section IV of Table I.

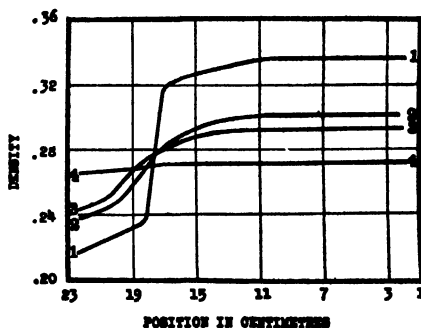


FIG. 4. Shows the relation between density and position in the bomb for four different temperatures in the region of the critical temperature. Curve No. 1 corresponds to section I, curve No. 2 to section II, curve No. 3 to section III and curve No. 4 to section IV of Table II.

Table I was compiled from observations on bomb No. 9. The conditions of heating and the position of the density value recorded are noted in connection with each section of the table.

Table I shows the variations in the density values at positions well above and well below the meniscus, while the temperature is raised or lowered through the point where the meniscus vanishes. Table II shows how the densities are distributed throughout the length of the bomb while the temperature is maintained constant. Again, bomb No. 9 is the one chosen to illustrate this point. The meniscus became invisible to the eye at  $126.9^{\circ}\text{C.}$ , at a point 18 cm. from the bottom of the bomb.

TABLE II  
DISTRIBUTION OF DENSITIES IN BOMB NO. 9 AT VARIOUS TEMPERATURES

I		II		III		IV	
Temp., $127.1^{\circ}\text{C.}$		Temp., $126.7^{\circ}\text{C.}$		Temp., $125.5^{\circ}\text{C.}$		Temp., $135.1^{\circ}\text{C.}$	
Position, cm.	Density	Position, cm.	Density	Position, cm.	Density	Position, cm.	Density
22.6	.2421	22.6	.2384	22.1	.2178	23.2	.2657
20.5	.2560	20.2	.2454	21.0	.2253	20.6	.2685
18.4	.2724	18.1	.2713	19.7	.2290	17.0	.2713
16.2	.2847	16.1	.2867	18.6	.2329	15.5	.2713
12.1	.2925	12.5	.2985	16.6	.3201	12.2	.2729
7.6	.2930	9.2	.3009	12.7	.3305	8.8	.2725
2.0	.2934	5.7	.3004	10.9	.3326	5.4	.2720
		2.2	.3006	8.6	.3363	1.6	.2727
				5.7	.3358		
				1.8	.3360		

NOTE: The numbers appearing under the heading "position" denote the cathetometer reading corresponding to the upper tip of the float. The bottom of the bomb on the same scale is equivalent to a reading of  $-1.2\text{ cm.}$

The temperature was equalized throughout the bath. The contents of the bomb was well stirred. See Fig. 4 for graph.

From a graph similar to that of Fig. 4, but containing lines for two more temperatures, Table III was compiled.

TABLE III  
DISTRIBUTION OF DENSITIES IN BOMB NO. 9 AT VARIOUS TEMPERATURES

Temp., $^{\circ}\text{C.}$	Density at 22 cm.	Density at 2 cm.	Diff.	Temp., $^{\circ}\text{C.}$	Density at 22 cm.	Density at 2 cm.	Diff.
125.5	.2200	.3360	.1160	128.9	.2580	.2800	.0220
126.7	.2390	.3010	.0620	132.3	.2650	.2730	.0080
127.1	.2450	.2940	.0490	135.1	.2680	.2720	.0040

NOTE: See Fig. 5 for graph of this table.

The influence of a temperature gradient along the length of the bomb is shown clearly by a consideration of the following results:

TABLE IV  
INFLUENCE OF A TEMPERATURE GRADIENT ALONG THE LENGTH OF THE BOMB

I				II			
Temp., °C.	Density	Temp., °C.	Density	Temp., °C.	Density	Temp., °C.	Density
118.4	.1440	126.1	.271	124.4	.3580	126.4	.2585
120.9	.1600	126.4	.271	124.8	.3491	126.6	.2596
125.5	.1724	127.0	.271	125.4	.3323	127.0	.2596
124.1	.1926	128.7	.271	125.8	.3161	129.7	.2616
125.9	.2467	130.9	.270	126.0	.2585	130.9	.2629
126.0	.2655	133.3	.270	126.2	.2585		

NOTE: I, the float was at a position above where the meniscus disappeared; II, the float was at a position below where the meniscus disappeared. In both I and II the bottom of the bomb was slightly warmer than the top ( $\frac{1}{10}$ °C.). See Fig. 6 for graph.

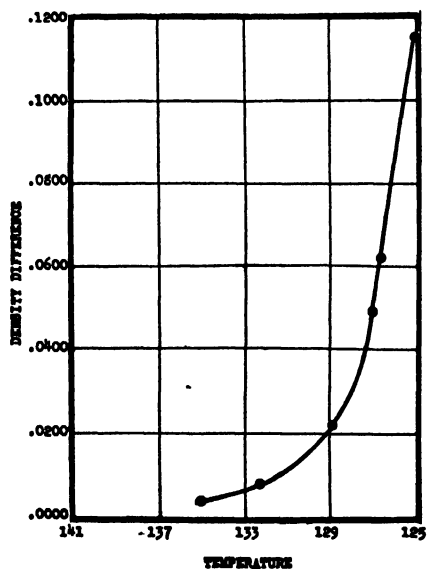


FIG. 5. Shows the relation between the difference in density above and below the position of meniscus disappearance and the temperature. (Corresponds to Table III.)

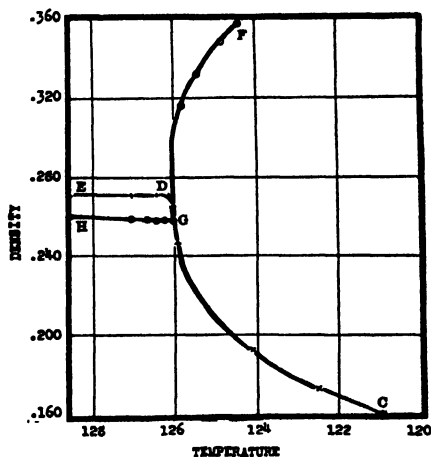


FIG. 6. Shows the relation between density and temperature in the critical region when the bottom of the bomb is  $\frac{1}{10}$ °C. warmer than the top. Curve FGH corresponds to section I and curve CDE corresponds to section II of Table IV.

The complete reversal in the existing density conditions brought about by the slight temperature excess at the bottom is so striking that unless confirmation had been supplied by repeated trials on many bombs, the results would not have been thought reliable. So interesting was this phase of the work that much time was spent upon it and no amount of stirring or agitation within the bomb was ever found that could materially alter the above-outlined



state of affairs. Arising out of the previous work came the thought that possibly the oft-repeated differences in density obtained with equalized temperature throughout were caused by an equilibrium lag between the two phases since the conditions could be so easily reversed by the appropriate temperature gradient.

Table IVa shows the result of maintaining a bomb at a temperature slightly above the critical temperature for a prolonged period of time, accompanied by continuous and vigorous stirring within the bomb.

From this table it will be seen that apparently a steady state of affairs has been arrived at and the density above the position of disappearance of the meniscus is still less than below.

TABLE IVa  
INFLUENCE OF TEMPERATURE HELD SLIGHTLY ABOVE THE CRITICAL

Time, min.	Position	Density	Time, min.	Position	Density	Time, min.	Position	Density
60	Bottom	.2899	160	Top	.2585	240	Bottom	.2798
90	Bottom	.2865	165	Top	.2590	255	Bottom	.2814
105	Bottom	.2865	180	Top	.2606	260	Bottom	.2814
120	Bottom	.2865	195	Top	.2618	270	Top	.2611
150	Bottom	.2865	210	Top	.2627	280	Top	.2618
153	Top	.2576	225	Top	.2634	290	Bottom	.2805
			235	Top	.2646	305	Top	.2632
						345	Top	.2646

NOTE: Bomb No. 9. Temperature 127.9° C., temperature equalized.

To show the result of a succession of temperature gradients upon any one bomb at some temperature slightly above the critical temperature, consider the following tabulation:

TABLE V  
INFLUENCE OF A SUCCESSION OF TEMPERATURE GRADIENTS AT TEMPERATURE SLIGHTLY ABOVE THE CRITICAL

Series no.	Gradient	Density diff.	Time, min.	Series no.	Gradient	Density diff.	Time, min.
1	(i) Bottom + $\frac{1}{8}^{\circ}$ C.	0	44	4	(i) All equalized	.0281	100
	(ii) All equalized	0	40		(ii) Bottom + $\frac{1}{8}^{\circ}$ C.	0	75
2	(i) All equalized	.0275	85		(iii) Top + $\frac{1}{8}^{\circ}$ C.	.0139	120
	(ii) Top + $\frac{1}{8}^{\circ}$ C.	.0402	45		(iv) All equalized	0	35
	(iii) All equalized	.0257	20	5	(i) Bottom + $\frac{1}{8}^{\circ}$ C.	0	140
	(iv) Bottom + $\frac{1}{8}^{\circ}$ C.	.0026	55		(ii) All equalized	0	28
3	(i) Bottom + $\frac{1}{8}^{\circ}$ C.	0	100		(iii) All equalized	0	26*
	(ii) All equalized	0	75				
	(iii) Top + $\frac{1}{8}^{\circ}$ C.	.0184	10				

\*While the average temperature was lowered from 127.9° to 126.4° C.

NOTE: Bomb No. 9, average temp. of bath 127.9° C. Critical temperature (disappearance of meniscus) 126.9° C.

To leave no doubt as to the meaning of the data in Table V, one of the series of experiments (Series No. 2) is described at some length.

The temperature was raised from room temperature to a point  $1^{\circ}\text{C.}$  above the critical temperature with a uniform distribution of temperature throughout the bath. (*All equalized.*) After 85 min. the density difference was 0.0275. The average temperature of the bath being kept constant, a temperature gradient was maintained so that for 45 min. the top was  $\frac{1}{10}^{\circ}\text{C.}$  higher than the bottom; under these conditions the density difference was increased to 0.0402. Then upon *equalization* of the temperature, after 20 min. the density difference returned to about the original value, 0.0257. When the *bottom* of the bomb was maintained at a  $\frac{1}{10}^{\circ}\text{C.}$  warmer than the top, the density difference after 55 min. was found to be only 0.0026.

Keeping a temperature gradient such that the bottom of the bomb is warmer than the top, even by a slight amount, effectively does what any amount of stirring over a much longer period of time at equalized temperature fails to accomplish, namely, the removal of the density difference.

#### *A Note on the Nature of the Fog*

Under the conditions imposed in section (iii), Series No. 5, of Table V, the formation and disappearance of the fog presented a remarkable sight. The tube remained perfectly clear down to  $126.5^{\circ}\text{C.}$ ; at  $126.4^{\circ}\text{C.}$  a mist flashed into view throughout the entire length of the bomb. The intensity was uniform and the distribution complete. As the temperature dropped, the mist increased in intensity, and at  $126.3^{\circ}\text{C.}$  entirely concealed every trace of the spiral and float. Suddenly a clear spot appeared immediately at the bottom of the bomb. This clear spot swept upward at about 1 cm. per sec. Simultaneously a clear region fell, with equal rapidity, from the top. The clear regions met about 12 cm. from the bottom and revealed a sharp meniscus. The whole dissipation of the fog occupied not more than 15 sec. It will be noted that the temperature of the reappearance of the meniscus was about  $0.5^{\circ}\text{C.}$  lower than that of its disappearance.

Table VI shows the way in which the density difference between the upper and lower portions of the bomb varies with respect to temperature in a number of different bombs. In all cases the temperature in the bath had been equalized throughout the length of the bomb, and a period of time sufficiently long had been allowed for apparent equilibrium to have become established. The tem-

TABLE VI  
COMPARISON OF DENSITY EFFECTS IN DIFFERENT BOMBS

Temp., $^{\circ}\text{C.}$	Density difference		
	Bomb No. 9	Bomb No. 10	Bomb No. 13
0.1	—	—	0
0.2	0.0490	0.0585	0
1.0	.0300	.0187	0
2.0	.0220	.0067	0
5.4	.0080	.0020	0

NOTE: Height of meniscus above the bottom of the bomb at the moment of disappearance of the meniscus—bomb No. 9, 18 cm.; bomb No. 10, 4 cm.; bomb No. 13, 11.2 cm

perature values listed are in terms of degrees Centigrade *above* the temperature at which the meniscus became invisible. The relative amounts of liquid in each bomb may be judged from the height at which the meniscus vanished.

A survey of Table VI shows that the three bombs do not agree on a number of points, the chief one being that No. 13 exhibits no density difference immediately above the critical temperature. This was the only bomb constructed which behaved in this way and a possible explanation lies in the fact that the proportion of liquid to available space in this particular bomb was unique. A diagram (Fig. 7) has been prepared to show just how the three bombs differed with respect to the quantity of liquid used in each case, and also to show how this quantity of liquid rose or fell up to the critical temperature. It must be understood that the rate of heating could modify the particular shape of any one of the curves, but a fair representation has been made under a slow rate of heating. For reference purposes, the curves may be reduced to their respective tangent values.

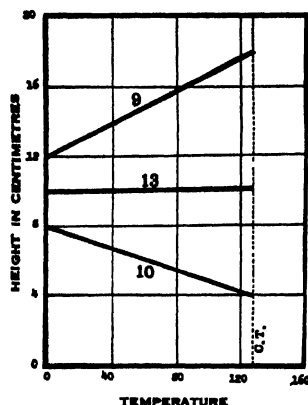


FIG. 7. Shows graphically the behavior of the meniscus in three different bombs under the influence of heating.

three bombs differed with respect to the quantity of liquid used in each case, and also to show how this quantity of liquid rose or fell up to the critical temperature. It must be understood that the rate of heating could modify the particular shape of any one of the curves, but a fair representation has been made under a slow rate of heating. For reference purposes, the curves may be reduced to their respective tangent values.

Curve No. 9 for bomb No. 9,  $\tan = +\frac{1}{2}$ ; Curve No. 10 for bomb No. 10,  $\tan = -\frac{1}{2}$ ; Curve No. 13 for bomb No. 13,  $\tan = 0$ .

It may be seen from the preceding paragraph that the three bombs under consideration differed from one another, in construction, in one important factor. In bomb No. 9 the meniscus rose steadily up to its point of disappearance, in bomb No. 10 it

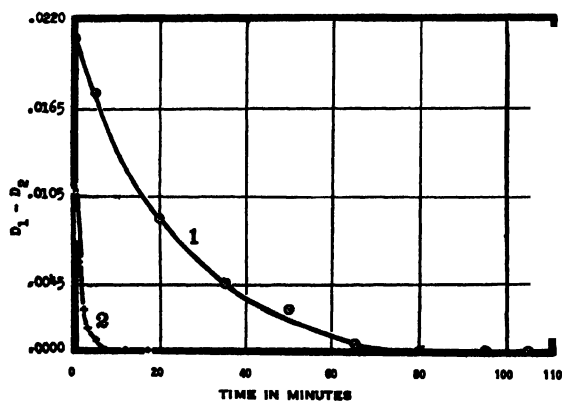


FIG. 8. Shows the rate with which the density of the vapor approaches its final value (no stirring) at two different temperatures above the critical.  $D_1$  is the density of the gas at infinite time.  $D_2$  is the density of the gas at the end of time  $T$ . Curve No. 1 was made at a temperature of  $127.1^\circ \text{C}$ , i.e.,  $0.2^\circ$  above the critical temperature. Curve No. 2 was made at a temperature of  $132.3^\circ \text{C}$ , i.e.,  $5.4^\circ$  above the critical temperature.

fell steadily, and in bomb No. 13 it neither rose nor fell. This last condition is very difficult to duplicate and was not met with in any other bomb constructed throughout this work. Since the same bomb which fulfilled this peculiar condition also exhibited no density difference between the top and bottom above the critical temperature, it might be logical to assume that the diversions were inter-related. The purity of the methyl ether and its manner of addition to the bombs were identical in every case.

The following tables have been compiled with a view to showing

TABLE VII

EFFECT OF NO STIRRING WITHIN THE BOMB  
ON FINAL DENSITY VALUE

*Time, (t) min.	$D_1 - D_2$	**Time, (t) min.	$D_1 - D_2$
0	0.0215	0	0.0110
5	.0177	1	.0061
20	.0090	2	.0028
35	.0046	3	.0015
50	.0028	5	.0007
65	.0005	7	.0000
80	.0000	12	.0000
95	.0000	17	.0000
105	.0000		

NOTE: Bomb No. 13.  $D_2$  was the density at time  $t$ .  $D_1$  was the density at infinite time, i.e., the highest density value obtained.

\*Temp.  $0.2^\circ$  above the critical. No stirring within the bomb.

\*\*Temp.  $5.4^\circ$  above the critical. No stirring within the bomb.

TABLE VIII

EFFECT OF STIRRING WITHIN THE BOMB ON  
FINAL DENSITY VALUE

*Curve 3, Fig. 9		**Curve 4, Fig. 9	
Time, min.	$D_1 - D_2$	Time, min.	$D_1 - D_2$
0	0.0208	0	0.0136
3	.0147	10	.0091
8	.0091	18	.0064
13	.0077	26	.0050
43	.0042	35	.0035
88	.0031	50	.0031
133	.0013		
163	.0000		
178	.0000		
193	.0000		
208	.0000		

\*Temp.  $0.2^\circ$  C. above the critical. No stirring within the bomb. Temperature equalized.

\*\*Temp.  $0.2^\circ$  C. above the critical. Continuous stirring within the bomb. Temperature equalized. See Fig. 9.

the manner in which the density in the gaseous phase approaches its final value when uninfluenced by any stirring. Two different temperatures have been selected to exhibit this, one only a short distance above the critical temperature, and the other a considerable distance above. In neither case

was a meniscus nor any discontinuity apparent to the eye.

In Table VII the value 0.2538 was taken as the gaseous density at infinite time. This value was arrived at by maintaining the bomb at either of the two temperatures for a prolonged period of time. Furthermore, this was also the density value for the lower portion of the bomb and might well be considered as the mean critical density for this particular tube. See Fig. 8.

Table VIII has been compiled to show the effect of stirring within the bomb upon the rate with which the density value of the gaseous medium

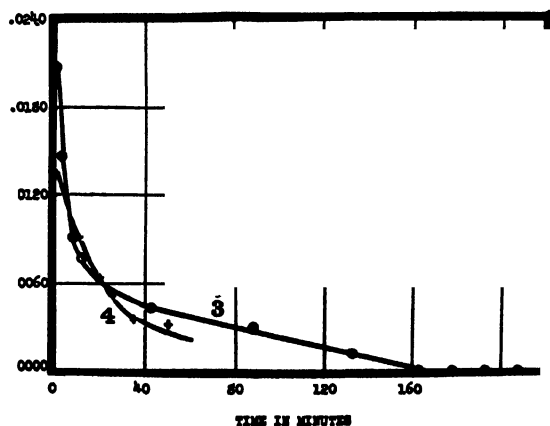


FIG. 9. Shows the small influence which stirring has upon the rate with which the vapor reaches its final density value.  $D_1$  is the density of the gas at infinite time.  $D_2$  is the density of the gas at the time  $T$ . Curve No. 3 was made at a temperature of  $127.1^\circ$  C., i.e.,  $0.2^\circ$  above the critical temperature. No stirring was performed within the bomb during the time these results were being obtained. Curve No. 4 was made at a temperature of  $127.1^\circ$  C., i.e.,  $0.2^\circ$  above the critical temperature. The contents of the bomb was well stirred during the time these results were being obtained.

approached its equilibrium value. The data were obtained from bomb No. 10 in which the density of the upper portion of the tube never reached as high a value as that in the lower. The value chosen to represent  $D_1$  was the largest value ever obtained in any run with the temperature equalized. The figures for curve No. 4, Fig. 9, were not obtainable past the point indicated on the graph, but the general trend does not appear to be radically different from that in curve No. 3. This relatively close agreement between the two curves seems to indicate that diffusion alone could not have been the cause for the slow adjustment of the density to its final equilibrium value.

### Summary

To illustrate the general nature of the results in a condensed form, the following brief summary has been appended. The conclusions listed here, have been based upon data of the type cited in this paper.

(1) The density above and below the position of the disappearance of the meniscus is not the same for several degrees above the temperature at which the meniscus becomes invisible.

(2) The generalization expressed in (1) above, applies to experimental tubes filled to such an extent with liquid that the meniscus *either* rises or falls when the temperature is raised slowly from room temperature to the critical temperature. The unique condition where the meniscus neither rises nor falls seems to influence this density difference to such an extent that it completely disappears immediately at the critical temperature.

(3) A slight temperature gradient along the length of the bomb during the time of density measurements greatly alters the results. Sections (1) and (2) above are true under conditions of equalized temperature along the entire length of the bomb, checked to within  $\frac{1}{10}^{\circ}$  C.

(4) By artificially creating a temperature gradient, such that the bottom of the bomb is about  $\frac{1}{10}^{\circ}$  C. warmer than the top, the density within will equalize over a period of 40 to 50 min. while the same bomb at equal temperature top and bottom will not show any indication of equalizing the density over periods of four or five hours accompanied by vigorous stirring.

(5) By increasing the temperature at the bottom by  $\frac{1}{2}^{\circ}$  C. over that at the top, a complete reversal of densities will be obtained in which the medium at the top has apparently a greater density than the material at the bottom. This condition will maintain itself in spite of vigorous stirring of the contents for indefinite periods of time.

(6) By artificially causing the top of the bomb to become heated to  $\frac{1}{2}^{\circ}$  C. warmer than the bottom, the condition outlined in Section (1) will be accentuated.

(7) Once a condition of equal density throughout the bomb has been obtained in the manner outlined above, then a return to uniform temperature will not in any way alter the density conditions within the bomb.

(8) Upon *slowly* cooling the entire bath when a condition of uniform density prevails, the fog formation is exceptionally pronounced and no meniscus appears until the temperature has fallen about  $\frac{1}{2}^{\circ}$  C. below that at which the meniscus disappeared with a rising temperature.

(9) Vigorous stirring of the contents has no effect upon the final density obtained and very little effect upon the rate with which it is reached.

(10) The rate at which the equilibrium density value is approached increases markedly with the extent to which the temperature is in excess of the critical value.

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# THE DISCONTINUITY IN THE ADSORPTION OF GASES, VAPORS, AND LIQUIDS ON SOLID SURFACES AT THE CRITICAL TEMPERATURE UNDER CRITICAL PRESSURE: SYSTEM PROPYLENE-ALUMINA<sup>1</sup>

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## Abstract

An apparatus and a technique for studying the adsorption of gases, vapors and liquids on solid surfaces are described. The arrangement permits investigations in the region of the critical temperature and the critical pressure. Results with the system propylene and alumina are given. Adsorption from the gas and vapor phases indicates the formation of a surface complex which is unstable at low pressure and high temperature. The density of the adsorbed phase is greater than that of the bulk phase. There is no discontinuity in adsorption processes with a change from vapor state to gaseous state. No evidence was obtained of an increase in critical temperature on the surface of the solid. Adsorption does not occur from the liquid state, and there is a marked discontinuity in the adsorption curve with a change from liquid state to gaseous state. This is probably due to a change in the forces of attraction between liquid molecules and the solid as compared with the attraction between gaseous or vapor molecules and the solid surface. If this is the case it is further evidence for the discontinuity in the region of the critical temperature, which has been previously observed in other experiments in this laboratory.

## Introduction

The sorption of gases, vapors and liquids upon solid surfaces has been extensively studied but in the majority of cases the measurements have been made at relatively low pressures, owing primarily to the experimental difficulties involved in all work under pressure. As evidenced by a recent symposium upon adsorption (6), there is much controversy and uncertainty regarding some of the most fundamental concepts of the phenomena. This is particularly true of the sorption of vapors and the nature of the adsorbed phase. Hence it is evident that any system which permits an examination of these problems should yield results of theoretical significance. The main reason, however, for the investigation to be described was to discover whether there exists a discontinuity, at the critical temperature and under the critical pressure, in the properties of a substance, such that this discontinuity might be made apparent by sorption measurements.

The possibility of the existence of a discontinuity of state under critical temperature conditions seems necessary to explain the discontinuity of reaction velocity discovered in this laboratory (21). A whole series of investigations was started in consequence of this work. One by Winkler and Maass (25) and another by Tapp, Steacie and Maass (22) have been published, and a third by Winkler and Maass will appear shortly. In these papers the question of "discontinuity" and its consequences on some of the properties of a system are referred to in detail.

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### Experimental

The apparatus of McBain and Britton (14), while admirably adapted for their purpose, could not be used by the writers in the form described. It was necessary for the object in view to have greater flexibility in immersing the sorbent at will in either compressed vapor or compressed liquid of the sorbate. Furthermore the temperature of liquid and vapor had to be kept at definite values without an intermediate indeterminate region. Hence although the success of the experiments was altogether dependent on that excellent instrument, the McBain-Bakr balance (13), the experimental arrangement was different from that of McBain and Britton and therefore deserves a somewhat detailed description. This is especially so since the results will have to be evaluated in the light of the density determinations of Winkler and Maass (26) and their data can be applied only to the form of apparatus described by the authors.

#### *The Selection of a System for Study*

There are a large number of solids which have been extensively studied as adsorbents, of which charcoal, silica and alumina are the most important. In view of the fact that one purpose of this study was to determine the effect of pressure, alumina was the solid employed. The selection of a sorbate which could vary through the phases of gas, vapor and liquid within a reasonable temperature range, and at pressures consistent with their use in glass bomb tubing, had been previously made in this laboratory. Propylene with a critical temperature of  $92.3^{\circ}\text{C}$ . and a critical pressure of 46.2 atm. seemed to be the most suitable for study in this apparatus. Dohse and Kälberer (5) studied the system propylene-bauxite from  $0$ – $120^{\circ}\text{C}$ . but up to pressures of only 5 mm.

#### *Description of Apparatus*

The apparatus to be described permits a study to be made of the above system over a wide range of temperature in so far as the temperatures of both the gel and the phase being studied are concerned, and can be varied to a considerable degree. It also permits an actual study of sorption in the condensed phase, after the gel has been completely immersed in liquid. It is also believed that this system presents a much more effective method of temperature control than that of McBain and Britton (14), a factor which is of utmost importance in such studies. Furthermore it would seem that, in view of the known heat conductivity of thick Pyrex glass, the absolute control of the temperature would be rather difficult in the method used by these workers. In such a system a very slight temperature gradient along the bomb would markedly affect the pressure in the system.

The arrangement of the apparatus is shown in Fig. 1. The bomb *ABC* was made of special Pyrex bomb tubing with a bore of 12 mm. and 2–3 mm. wall thickness. A perforated aluminium basket was filled with alumina and suspended from the bottom hook of a carefully calibrated quartz spiral.



The spiral was hung by a glass hook from a Pyrex stirrup, held in the tube by small indentations in the wall of the bomb. The arm containing this suspension was connected by a capillary tube to the bomb containing the propylene to be adsorbed. This capillary *B* was wound with resistance wire

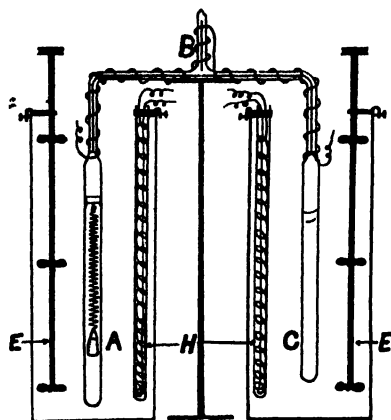


FIG. 1. Diagrammatic sketch of high pressure sorption apparatus.

to maintain the glass at a temperature slightly above that of the propylene to prevent condensation of the latter. A thermometer bent at right angles was placed between the wire and the capillary to determine the temperature of the latter. In what follows the side containing the suspension will be designated the gel side, and that with the liquid will be called the bomb side.

The bath surrounding the gel side consisted of a large Pyrex jar which may be filled with any suitable liquid, transparent enough to permit readings of the extension of the spiral with a cathetometer. Butyl phthalate was found to be the most suit-

able for this purpose as it did not darken at high temperatures.

As a precautionary measure the bath surrounding the bomb side was made from a 6-in. iron pipe, lagged to prevent heat radiation. For low pressure studies the temperature of the propylene was maintained by carbon dioxide-acetone mixtures in a Dewar flask. This permitted very exact control of temperatures. The large baths were electrically heated as shown at *H*. It was necessary that there be no temperature differential in the baths hence the stirrers *E* had to be very effective. The temperature of the gel bath was read with a calibrated thermometer, while that of the bomb was read with a five-junction chromel-alumel thermocouple.

There is considerable danger attached to the study of high pressure phenomena in glass tubing, and for this reason the whole apparatus was enclosed in a heavy wooden case, strapped with band iron, and containing two windows of shatter-proof glass. The cathetometer for measuring the extension of the spiral was placed about four feet from the apparatus behind another sheet of heavy plate glass.

Thus an apparatus has been devised which permits the study of the sorption under pressure of gases, vapors and liquids. By the elevation of the temperature of the sorption bath above the critical temperature of the phase being sorbed, it became a study of gaseous sorption regardless of the pressure. Below the critical temperature, at pressures corresponding to the vapor pressure of propylene, the system becomes a vapor sorption study; while by raising the vapor pressure of the propylene, liquid may be condensed on and around the gel and the latter completely immersed if so desired.

### *Preparation for an Experiment*

The spiral used in these experiments had a sensitivity of 0.00365 gm. per mm. and a maximum load of about 0.5 gm. Obviously the weight of the gel plus pan plus adsorbate could not exceed this latter value. The gel was prepared as described by Munro and Johnson (15), and a consideration of their results together with those of Perry (18) indicated that a maximum increase in weight of about 25–35% might be expected. Alekseevskii (1) found that alumina ignited to 600–620° C. gave the optimum sorption of ethylenic hydrocarbons; this gel was heated to 600° C. in a muffle furnace. It was then placed in the bomb, and the latter was connected through the capillary *B* to a high vacuum pump and the gel again heated to 400° C. under high vacuum. In this operation care was taken not to expose the spiral to this temperature in view of the observation of McBain and Britton (14) that the sensitivity of the spiral was changed upon subjection to such a temperature.

Pure propylene was prepared by the dehydration of isopropyl alcohol over alumina at 300° C. and purified by low temperature fractionation as described by Maass and coworkers (3, 10, 11). The purity of the propylene was checked by vapor pressure measurements during fractionation. The bomb was evacuated and the system flushed several times with propylene vapor, following which liquid propylene was condensed in the bomb using a carbon dioxide-acetone mixture, and the capillary was sealed off.

Zero sorption was found by freezing the propylene in liquid air while the gel was maintained at 95° C. The actual weight of the gel was found to be 0.32957 gm.

### **Experimental Results**

The presentation of the results requires very little comment. Sorption is indicated in the usual form of  $x/m$ , in which  $x$  is the weight of material adsorbed and  $m$  the weight of the gel. This factor shows the weight of propylene sorbed per gram of alumina. With the exception of the region in which condensation to the liquid phase was approaching, the sorption equilibrium seemed to be attained almost immediately. In all cases however the readings were checked for a period of time to ensure a constant value. Points on the isobars were checked occasionally to be certain the zero value of the gel was not changing with time.

Pressure was determined by the vapor pressure of propylene. At low temperatures the values of Maass and Wright (10, 11) were used; at higher temperatures the data of Francis and Robbins (8) were used, as it checked with recent determinations in this laboratory.

Since this was a gravimetric method the density of the medium surrounding the suspension was of vital importance in correcting for buoyancy. The values at high pressures were particularly well known from the work of Winkler and Maass (26) and in this region the corrections were of greatest magnitude. At lower temperatures the factor was not as large but the values

were not as well known. Data of Ormandy and Craven (16) were employed and points in the intermediate range found by interpolation of the curve. One assumption was made, *i.e.*, that the density varied normally over short temperature ranges when the pressure was constant. To some extent this may be unjustified but the deviation would be too small to materially alter the ensuing calculations.

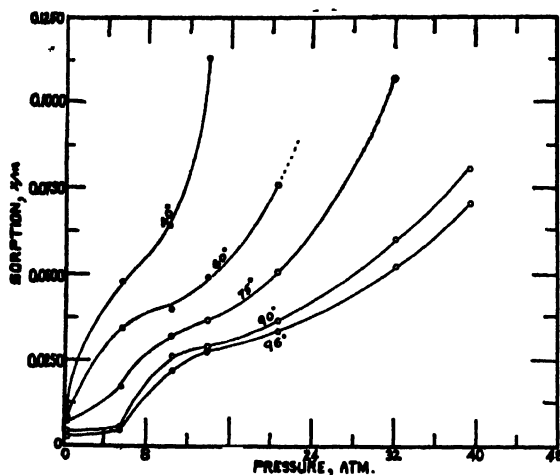


FIG. 2. Isotherms showing the adsorption of propylene gas and vapor on alumina. Temperatures in °C.

### Vapor-gas Media Surrounding Sorbent

It was most convenient to follow the process of sorption of gas and vapor by determining the isobars at increasing and decreasing temperatures of the sorbent, but one isotherm was determined to prove the adaptability of the system. The results are shown in detail in Tables I and II and more clearly in Figs. 2 and 3. The values in Table I have been selected from 190 readings for convenience in tabulation and indicate the course of the process quite fully. In Table II a few selected values

indicate the magnitude of buoyancy corrections and show clearly the necessity of an accurate knowledge of the density of the phase, particularly at high pressures. In all cases the gel and the pan were considered together as sorbing material.

TABLE I

SORPTION OF VAPOR AND GAS ON GEL AT VARIOUS TEMPERATURES AND PRESSURES

Gel temp., °C.	Pressure—atm.						
	0.2	5.7	10.5	13.9	20.7	32.1	39.4
	Sorption, %/m						
10	0.0291	0.0812					
20	0.0239	0.0591					
30	0.0156	0.0527	0.1372				
40	0.0130	0.0476	0.0596	0.1140			
50	0.0099	0.0402	0.0492	0.0656			
60	0.0076	0.0322	0.0396	0.0484	0.0746		
70	0.0078	—	0.0339	0.0387	0.0530		
75	—	0.0170	0.0318	0.0361	0.0492	0.1037	
80	0.0076	—	0.0298	0.0351	0.0454	0.0822	
84	—	—	—	—	0.0437	0.0697	
87	—	0.0086	0.0275	0.0304	0.0387	0.0640	0.0867
90	0.0041	0.0059	0.0265	0.0285	0.0361	0.0597	0.0794
93	0.0028	—	0.0233	—	—	0.0557	0.0742
97	0.0029	0.0047	0.0218	0.0269	0.0329	0.0512	0.0629
102	—	—	0.0192	0.0240	0.0307	0.0482	0.0585
107	—	—	0.0186	0.0228	—	0.0451	0.0536

TABLE II

INFLUENCE OF BUOYANCY AND ITS CORRECTIONS WITH INCREASING TEMPERATURE AND PRESSURE

Gel temp., ° C.	Vapor press., atm.	Apparent $\alpha/m$	Vapor density, gm./cc.	Buoyancy correction	True $\alpha/m$
19.7	0.2	0.0242	0.00035	0.0001	0.0243
29.7	5.7	0.0502	0.0094	0.0028	0.0530
38.0	10.5	0.0618	0.0170	0.0051	0.0669
47.7	13.9	0.0675	0.0238	0.0071	0.0746
63.7	20.7	0.0546	0.0374	0.0112	0.0658
82.4	32.1	0.0485	0.0808	0.0242	0.0727
90.2	39.4	0.0438	0.1172	0.0352	0.0790

*Liquid-gas Media Surrounding Sorbent*

The results obtained in the liquid-to-gas change require some elaboration. Two methods were employed: in the first, the bomb was held at a fixed temperature, 94° C. in one case and 100° C. in another, the gel was immersed in liquid and the temperature was raised slowly through the critical value while readings of the extension of the spiral were made. In the other method the gel was maintained above the critical temperature and the bomb temperature was again raised to 94° or 100° C.

Tapp, Steacie and Maass (22) and Winkler and Maass (26) observed that above the critical temperature the density of the gas in the bomb was not continuous but was a function of the previous history of the bomb. Thus they found that the density of the medium below the point at which the meniscus disappeared was uniform, but greater than the density of the medium above this point, this also being uniform throughout. The above experimental procedure makes it possible to surround the sorbent with either of these phases at will. Typical calculations shown below illustrate this more clearly. It was anticipated that the rate of diffusion through the capillary might influence the rate of attainment of equilibrium values, particularly as the relative positions of the arms changed from top to bottom, these terms being used in a sense to correspond to top and bottom relative to the disappearance of the meniscus in a straight tube. Actually this lag was not particularly evident but might account for some of the slightly divergent points.

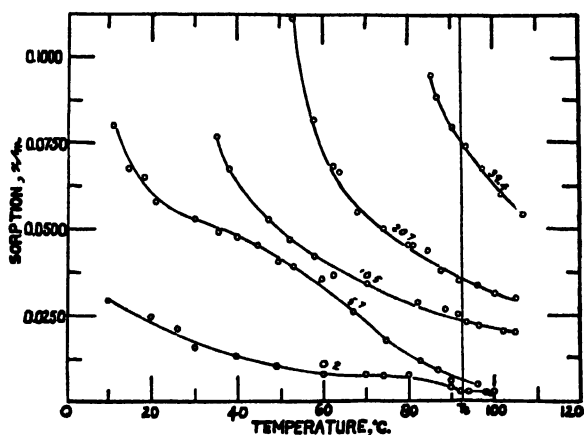


FIG. 3. Isobars showing the adsorption of propylene gas and vapor on alumina. Pressures in atmospheres.

Examples of the calculations indicate the importance of the density corrections. In these experiments the weight of the sorbent was 0.4121 gm. In both these examples the eventual bomb temperature was 100° C. but the relative position of the gel changed from the top to the bottom of the system. Thus in the first case the bomb was maintained at 100° C. and the gel was surrounded by liquid and the temperature raised to 94.6° C. The measured extension was 11.253 cm. corresponding to an apparent weight of 0.4107 gm. Since the gel was in the bottom of the bomb the density of the medium was 0.2130 gm. per cc., giving a correction of 0.0258 gm. and a net sorption of 0.0244 gm. or  $x/m$  equal to 0.0603.

In the second example the gel was maintained at 94.8° C. and the bomb raised from a lower temperature to 100° C. in which case the gel occupied the top of the system and the medium had a density of 0.2087 gm. per cc. The extension was 11.278 cm. corresponding to a weight of 0.4116 gm. The buoyancy correction was 0.0253 gm., giving a net sorption of 0.0248 gm. and  $x/m$  equal to 0.0613.

Selected values are shown in Tables III and IV and graphically in Figs. 4 and 5. In Fig. 5 Winkler's (26) density curve for propylene is plotted with

TABLE III  
SORPTION OF LIQUID AND GAS; BOMB AT 100° C.

Gel at bottom				Gel at top	
Temp., ° C.	Sorption, $x/m$	Temp., ° C.	Sorption, $x/m$	Temp., ° C.	Sorption, $x/m$
68.2	-0.0005	93.4	0.0467	94.8	0.0613
71.2	-0.0015	94.4	0.0531	95.0	0.0591
76.6	-0.0017	95.3	0.0561	96.2	0.0586
79.3	-0.0010	96.6	0.0593		
81.4	-0.0002	97.6	0.0620	93.9	0.0620
87.1	0.0002	98.0	0.0633	94.2	0.0566
91.2	0.0077	98.3	0.0655	94.8	0.0551
92.2	0.0175	101.8	0.0633	95.8	0.0588
92.5	0.0242	110.2	0.0640	97.8	0.0611
92.9	0.0371	115.8	0.0665	99.6	0.0626
				*92.7	0.0445
				*91.7	0.0405

\*Rapid cooling indicates a lag.

TABLE IV  
SORPTION OF LIQUID AND GAS; BOMB AT 94° C.

Gel at bottom				Gel at top			
Temp., ° C.	Sorption, $x/m$	Temp., ° C.	Sorption, $x/m$	Temp., ° C.	Sorption, $x/m$	Temp., ° C.	Sorption, $x/m$
88.0	-0.0022	92.7	0.0361	92.7	0.0704	107.5	0.0776
89.0	-0.0037	93.7	0.0628	94.8	0.0684		
90.1	0.0072	94.9	0.0756	96.6	0.0791	*95.3	0.0727
91.1	0.0040	96.0	0.0811	98.8	0.0786	*93.9	0.0702
91.7	0.0044	97.8	0.0808	101.2	0.0784	*92.8	0.0677

\*Rapid cooling shows lag.

the 94° C. sorption curve to illustrate the difference in type of curves, the significance of which will be discussed more fully later. It was observed that there were a few small irregularities, particularly when the gel was in the top of the system or on cooling. These were undoubtedly due to diffusion lags across the capillary but they were not of large magnitude. The hysteresis was not unexpected and the discrepancies practically disappeared with slow cooling.

There is one factor in the calculation of these curves which should be mentioned, that is, the influence of the change in bomb temperature from 94° to 100° C. It will be observed that the 94° C. curve shows a greater adsorption; this is due to the fact that at 100° C. the medium on the gel side would be more highly compressed and the buoyancy correction should be greater. Actually the influence of this compressibility was not known and

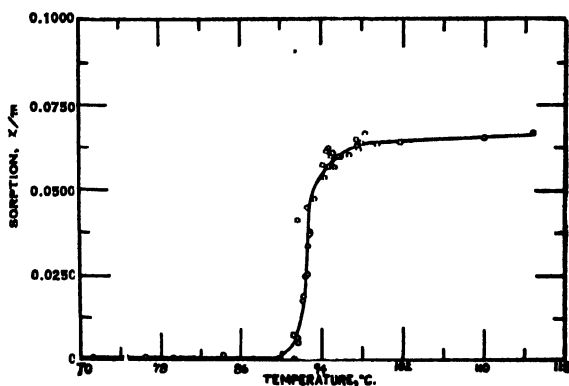


FIG. 4. Curve showing the discontinuity in the adsorption of propylene liquid and gas on alumina. Bomb temperature, 100° C.

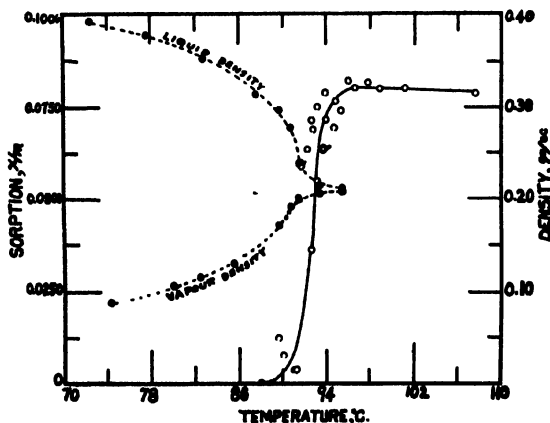


FIG. 5. Curves showing the discontinuity in the adsorption of propylene liquid and gas on alumina gel. Bomb temperature, 94° C., and compared with Winkler and Maass' curves for the density of propylene.

therefore the same densities were used in both cases. It is anticipated that the true densities will be determined before the completion of this research, but for the present purpose this does not alter the conclusions which are to be drawn. It is expected that with the application of the corrected density values these curves will practically coincide, thus it will be observed that the values when both sides are at 94° C. in the one case, or at 100° C. in the other, are practically the same, lending support to this concept. Furthermore, when the corrections are made the adsorption curves above the critical temperature will tend to slope downward more markedly and follow a normal gaseous isobar.

### Discussion

The interpretation of these results presents many points worthy of consideration at some length and the more important of these will be discussed

in detail. In the calculation of results the following equation was used:

$$W = w + d_m v_g - w_o,$$

where  $W$  is the weight of adsorbed material,  $w$  the apparent weight of the gel,  $d_m$  the density of the medium,  $v_g$  the volume of the gel, and  $w_o$  the zero weight of the gel. Obviously the factor  $d_m v_g$  represents the buoyancy correction.

It is at once evident that the volume of the adsorbed phase has not been considered. Hence the exact form of this equation should be developed as follows:

$$W_o = w + d_m v_g + d_a v_a - w_o,$$

where  $v_a$  is the volume of the adsorbed phase and  $W_o$  is the true weight of adsorbed material;  $v_a = \frac{W_o}{d_a}$ , where  $d_a$  is the density of this adsorbed phase.

Hence the true expression becomes,

$$W_o = \frac{w + d_m v_g - w_o}{1 - \frac{d_m}{d_a}}.$$

In this equation it is evident that if  $d_m = d_a$ , then  $w + d_m v_g - w_o$ , the measured value, becomes zero, hence  $W = 0$ , and furthermore  $W$  will have a positive value only when  $d_a > d_m$ . The meaning of this will be indicated later.

With regard to the density of the adsorbed phase, certain conclusions seem evident and these may remove some of the uncertainty regarding the nature of this phase. Thus Tryhorn and Wyatt (23) have measured the adsorption of organic vapors on porous substances and concluded from pore volume studies that the molecular volume of the adsorbed liquid was the same as that of the bulk liquid. On the other hand Dixon (4) assumed that the adsorbed gas condensed to a liquid whose density was greater than that of the normal liquid.

In studying the adsorption of a vapor above the liquid phase, if the adsorbed phase has the same density as the bulk phase, then adsorption would be measurable owing to the difference in densities of the media. However as the density of the vapor approached that of the liquid with a temperature increase, the adsorption curves should follow a form similar to that of the density curve and reach approximately a zero value at the critical temperature. It is evident that experimentally the latter is not the case and the adsorbed phase must have a greater density than that of the bulk phase. The density of the adsorbed phase, as represented by its concentration upon the surface, must decrease with temperature thus accounting for the form of the isobars.

#### *Vapor-gas Media Surrounding Sorbent*

The general form of the isotherms corresponds to an S-shaped curve similar to the adsorption of water vapor on cellulose. The interpretation of cellulose adsorption is that a surface corresponding to a monomolecular layer is formed with an increasing surface as sorption continues with a subsequent multi-

layer adsorption corresponding to the filling of capillary tubes (7, 9). In the cellulose-water vapor sorption curves the amount of adsorption at the same relative humidity is the same over a large temperature range. The same appears to be true of the isotherms of this alumina-propylene system, especially when account is taken of the fact that with the high pressure, and therefore high temperature isotherms, the true sorption values should be greater because the buoyancy of the adsorbed phase was not taken into account.

It is interesting to observe that the first part of the adsorption isotherms, which corresponds to a monomolecular layer forming a surface complex, requires increased pressure with a rise in temperature in order to be formed. There was no hysteresis which may be interpreted as an indication that there was no increase in surface with increased adsorption. Hence it would appear that the propylene first forms a surface compound with a subsequent filling of the capillaries, these latter portions of the curves being those in which the relative humidity relation holds. Consider the 40° and the 90° C. isotherms. In the former the S-shape starts at low pressure, in the latter the S-shape starts at about 5 atm. This is quite consistent with the idea that the first part of the adsorption process is a surface complex which dissociates at the higher temperature at low pressures and therefore requires the higher pressure values in order to be formed.

The form of the isobars also indicates this surface compound. At higher pressures the form indicates stability of the complex but the 5.7 atm. curve shows a marked change in form which is undoubtedly due to the high temperature dissociation of the surface form, which is quite stable at low temperatures and follows the normal form. It should be stressed that this curve was completely reproducible. The experiment was repeated several times and the curve plotted on each occasion to ensure that the form of the curve is as shown. At very low pressures the complex is not so stable but the slight discontinuities indicate its formation. Such curves have been obtained by other workers who also observed these reproducible discontinuities with other systems (2).

In the case of the cellulose-water vapor system, Filby and Maass (7) showed that water vapor sorbed on cellulose in the first part of the sorption isotherm has a density much greater than that of liquid water, assuming that the cellulose remains unchanged in density. Consequently it is not unreasonable to assume that in these curves the observed sorption in the first part of the curves at low temperatures corresponds to an adsorbed phase with a greater density than that of the bulk liquid under corresponding conditions. This point will be elaborated later.

It should also be pointed out that these curves are very different from those obtained under high pressure with charcoal as the sorbent. This is much as would be expected, according to McBain (12).

While the theoretical considerations of the behavior of vapors on solid surfaces have not been thoroughly elucidated, Rideal (20) and Polanyi (19)



have discussed the various factors and deduced two different forms of isotherm. The results with this system seem to favor the explanation of Rideal rather than that of Polanyi, but it would seem better at this stage to reserve a definite conclusion regarding this point.

It was stated that the main objective of this research was to investigate the question of a difference existing between molecules in the liquid and in the vapor or gaseous states, since the other investigations referred to indicated the existence of such a difference. No difference was to be expected however between molecules in the vapor or gas as defined by the critical temperature. That is, no discontinuity was to be expected in adsorption of gas or vapor at the critical temperature, but a discontinuity might be expected between adsorption from a liquid, and from a gas of density equal to that of the liquid. Whether the data obtained point to such a conclusion will now be discussed.

There is no discontinuity in adsorption in the change from vapor to gas as indicated quite fully by the regularity of the isobars (Fig. 3) through the critical temperature. Furthermore as shown by Fig. 2, isotherms plotted just above and just below the critical value are identical in form. It is thus evident that these may be considered as the same molecular species. The absence of an inflection in this regard has been interpreted by Patrick, Preston, and Owens (17) as indicative of an increase in the critical temperature of the sorbate in the capillaries. However in some of these cases the temperature was raised 15° C. above the critical value and it is unlikely that the capillaries could produce an effect of that order. Consequently there would seem to be no evidence for an increase in the critical temperature but rather it must be concluded that the adsorbed phase is not in the liquid or vapor state on the surface above the critical temperature.

#### *Liquid-gas Media Surrounding Sorbent*

The most striking feature of the change in adsorption from liquid phase to gas phase is the marked discontinuity at the critical temperature. There are several interesting facts revealed by a study of these curves. If no surface complex were formed with the liquid, and the adsorbed phase had the same density as the bulk phase, then obviously adsorption in such a system could not be measured. But this should also be true as the liquid phase changed to gas phase; in this latter case however sorption was measurable, indicating a difference in density between the bulk and adsorbed phases.

But if a complex were formed between the liquid and the sorbent it should be detectable (7). That this was not observed indicated that adsorption from the liquid phase did not occur, because as mentioned above, no adsorption means that  $d_m = d_a$  and this would not be compatible with the results obtained in the region of the critical temperature. Thus the densities of the medium just above and just below the critical temperature are not very different but the amount of adsorption is markedly increased.

If for the purposes of discussion we assume an equilibrium between molecules from the liquid and the adsorbed phase below the critical temper-

ature, and also an equilibrium between the molecules of the gas and the adsorbed phase above the critical temperature; then it is evident that there is a discontinuity in that equilibrium at the critical value, as there is no indication of adsorption from the liquid while the gas is strongly adsorbed. Hence the molecules of the liquid state are not the same as those of the gaseous state.

The above interpretation seems to be the most reasonable at the moment but is admittedly dependent on considerations involving the buoyancy of the adsorbed material. Further investigations on adsorption are to be carried out with a view to its confirmation. If this should be confirmed, then, since adsorption is not observed from the liquid phase, the attraction of liquid molecules of homopolar character is greater than the heteropolar attraction between the liquid and the sorbent. This discontinuity at the critical temperature is due to the change in intermolecular attraction and in this case the force of adsorption is greater than the inter-attractive forces between the gaseous molecules. This discontinuity in character of liquid and gas is in accord with the behavior that might be expected from the previous investigations in this laboratory.

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## THE EFFECT OF WINTER EXPOSURE IN THE STOOK ON QUALITY OF WHEAT<sup>1</sup>

BY R. K. LARMOUR<sup>2</sup>, J. G. MALLOCH<sup>3</sup> AND W. F. GEDDES<sup>4</sup>

### Abstract

Samples of wheat were exposed in the stook over winter and threshed in the spring in two seasons. These spring-threshed samples were compared with check samples from the same lots that had been threshed in the fall. The exposed samples lost grade in 50% of the cases, and decreased in weight per bushel in practically all cases. The flour yield was generally increased slightly as a result of the winter exposure. In respect to baking quality 22% showed improvement and 40% showed damage. The changes in grade and bushel weight do not correspond very closely with changes in baking behavior.

### Introduction

In some seasons, owing to unfavorable harvest conditions, a portion of the wheat in stook in western Canada becomes too wet to thresh and has to be left over winter and threshed in the spring. The percentage of this spring-threshed wheat is never large, but occasionally enough of it comes on the market to warrant question concerning its usefulness for milling and export.

This class of grain is characterized by bleached bran and starchy-appearing endosperm. The extent to which bleaching occurs will depend on the weather conditions during exposure and to some extent on the manner in which the grain is stooked. Under conditions necessitating spring threshing the grain will have been subjected to successive wettings and partial dryings, and the stooks, unless carefully made in the first place, may have settled badly with some of the sheaves lying on the ground. Such sheaves may undergo very serious damage from sprouting and from the growth of molds. The grain in the inside sheaves may escape damage altogether but on the other hand under some conditions it too may sprout or mold. When the weathering in the fall or spring has been severe enough to cause extensive sprouting or molding, the grain, if threshed, is generally considered unmarketable and usually is not offered for sale. If however late fall rains followed soon after by freezing weather is the cause of leaving the grain in the field for the winter, the drying in the spring is usually so rapid that little sprouting or mold damage is likely to occur. The appearance of the threshed grain is spoiled by the presence of a certain percentage of kernels which are more or less bleached. The effect of these is accentuated by contrast with the unbleached kernels in the sample. Furthermore the bleached grain when cut or broken shows a very white, starchy-looking interior similar to that of the soft winter wheats, and this

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similarity has given rise to the expression that such wheat "has gone starchy", the inference being that it has become degraded in strength.

It is extremely improbable that either bleaching or the change from vitreous to starchy appearance effects a material change in the strength of a wheat sample, provided other kinds of damage do not occur. Bleaching is concerned with the bran pigments and can be brought about by conditions that could not affect the strength. It is a common observation that ripe grain standing in the head awaiting the combine will undergo very severe bleaching from the effects of a light rain or even a heavy mist. In the ordinary process of tempering or conditioning preparatory to milling, much of the original bloom is lost and the grain may even appear somewhat bleached.

The change from vitreousness to starchiness seems to be associated with the fact that swelling occurs with wetting, and that this is not entirely reversible. Thus when wheat is brought to 18% moisture from a normal of about 11% the density, as indicated by bushel weight, decreases, and when the grain is dried back to its original moisture content of 11% the bushel weight does not come back to its original value but remains somewhat lower. This can be accounted for on the basis of the partial irreversibility of the swelling process, as a result of which larger air cells are left after drying. These air cells scatter the incident light and consequently the cross section appears whiter or less vitreous. Thus neither bleaching nor development of starchiness in themselves can be seriously considered as factors likely to affect in any way the inherent strength of a hard wheat, although it would appear quite possible that the latter change might be associated with some modification of the general colloidal characteristics of the endosperm. Saunders, Nichols and Cowan (4) showed that successive wettings resulted in an increase in loaf volume by the particular baking method used. This apparent increase in strength was also observed in a previous study of the effect of weathering on wheat (3) and was attributed to a sort of "mellowing" or "conditioning" effect which was apparent principally when the baking quality of the flour was tested by the simple formula. When, however, the bromate formula was used, this apparent improvement in quality nearly disappeared, indicating that no real change in inherent strength had occurred.

Despite these general conclusions regarding the likelihood of damage to strength by the factors discussed above, it was realized that commercial samples of spring-threshed grain might contain various percentages of severely damaged wheat, and it seemed desirable therefore to conduct some tests on samples that had undergone winter exposure under field conditions. To this end, samples of grain in the sheaf were collected in the fall of 1928 and of 1929. Each sheaf was divided carefully into two representative parts, one of which was threshed in the fall, the other being stooked in the field and left until spring. The samples obtained in this way will be referred to in this paper as the "fall-threshed" or "check" samples and the "spring-threshed" or "exposed" samples, respectively. The grain from each lot was divided into five-pound samples and sent to the collaborating laboratories for milling and baking tests.

## Methods

The milling tests were made according to the flow-sheet given by Geddes (1), a straight flour of approximately 94% extraction being used in all cases.

The baking tests were conducted according to the procedure described by Geddes, Malloch and Larmour (2). Five different baking formulas were used, namely: (1) the simple or basic formula—flour 100 gm., yeast 3 gm., sugar 2.5 gm., salt 1 gm., water as needed; (2) the bromate formula—as (1) plus 1 mgm. of potassium bromate; (3) the blend-bromate formula—as (2) but using a blend of 40% of club wheat flour and 60% of the flour under test; (4) the malt formula—the simple formula plus 1 gm. of Panomalt; and (5) the malt-Arkady formula—the simple formula plus 1 gm. of Panomalt and 0.5 gm. of Arkady.

In compiling the data, averages of the results were taken when the tests were made in more than one laboratory. Such average values discount to a large extent the personal factor always present in wheat testing technique and are more reliable than determinations made in a single laboratory. The data thus obtained are too bulky for inclusion in a paper of this sort and therefore we shall present only summary tables and charts.\*

## Experimental Results

### *Effect of Exposure on Official Grade*

Even though no damage to baking quality occurred, the bleaching of the grain might result in loss of grade which is a serious practical consideration. The data bearing upon this point can be summarized most readily by considering the numbers of samples in the various grades that were actually changed in grade as a result of exposure. These are shown in Table I. Of the 32 pairs of samples, three gained one grade, twelve were unchanged, nine lost one grade, and eight lost two grades. Of the eight samples that lost two grades, seven were No. 1 Northern in the fall.

TABLE I  
CHANGES IN GRADE DUE TO WINTER EXPOSURE

Fall-threshed		Spring-threshed			
Grade	No. of samples	Gained 1 grade	Unchanged	Lost	
				1 Grade	2 Grades
No. 1 Hard	1			1	
No. 1 Northern	15		5	3	7
No. 2 Northern	6	2	1	2	1
No. 3 Northern	8		5	3	
No. 4	—				
No. 5	1	1			
No. 6	1		1		
Total	32	3	12	9	8

\*The complete tables are on file with S. P. Eagleson, Secretary, National Research Council of Canada, and mimeographed copies are available to those interested in a minute study of the data.

*Effect of Exposure on Weight per Bushel*

The effect of exposure on weight per bushel is shown graphically in Fig. 1. Differences of less than one pound per bushel were not considered significant. On this basis six samples were unchanged in weight, fourteen lost 1 to 1½ lb., eight lost 2 lb. and three lost 3 lb. per bushel. One No. 3 Northern sample gained 1 lb. per bushel and lost one grade. Losses in weight were shown by 78%

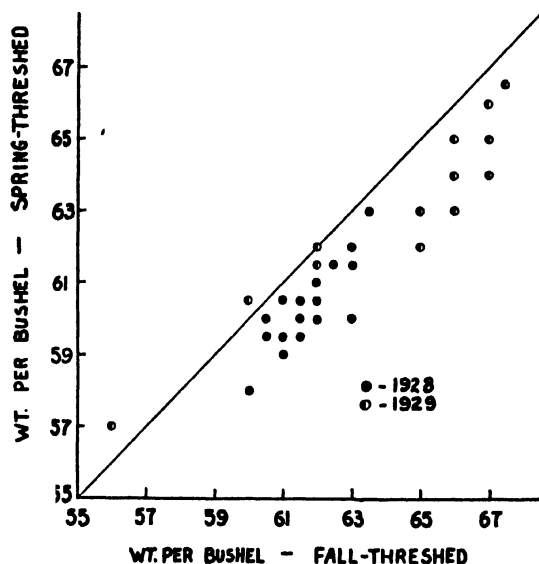


FIG. 1. Comparison of fall- and spring-threshed wheat in respect to weight per bushel.

of the samples and it may be concluded therefore that generally there can be expected a material lowering of bushel weight when wheat is exposed in the stook during the winter. Indeed it is surprising that they all did not show decreases since wheat once thoroughly wetted will not regain its former density on drying.

*Effect of Exposure on Flour Yield*

For the summary of flour yield, values obtained by calculating the yield of straight flour from the spring-threshed sample as a percentage of the yield from the check sample have been used. The distribution of these is shown in Table II.

TABLE II  
DISTRIBUTION OF VALUES FOR FLOUR YIELD

Fall-threshed		Spring-threshed as percentage of fall-threshed								
Grade	No. of samples	96-98	99	100	101	102	103	104	105	109
No. 1 Hard	1				1					
No. 1 Northern	15	1	2	2	1	4	2	2		1
No. 2 Northern	6	1	1		2	2				
No. 3 Northern	8	1	1		4	2				
No. 5	1			1						
No. 6	1								1	
Total	32	3	4	3	8	8	2	2	1	1

Twenty-two of the pairs gave higher flour yield from the spring-threshed than from the fall-threshed samples. This result is probably associated with the looser structure of the endosperm resulting from the successive wettings and dryings, a process which is somewhat analogous to conditioning prior to milling.

*Effect of Exposure on Baking Quality*

The absorption, or amount of water required to make the flour into a dough of the proper consistency, is important because it influences bread yield to a considerable extent. In this series the range of absorption was 62 to 68% and there were only two cases in which the spring-threshed samples showed higher values than the corresponding check samples. The distribution of the variations is given in Table III.

TABLE III  
DISTRIBUTION OF CHANGES IN ABSORPTION

Fall-threshed		Spring-threshed compared to the checks, variations in actual percentages				
Grade	No. of samples	+ 1	Unchanged	- 1	- 2	- 3
No. 1 Hard	1		1			
No. 1 Northern	15	1	6	4	3	1
No. 2 Northern	6		5		1	
No. 3 Northern	8	1	2	1	3	1
No. 5	1				1	
No. 6	1			1		
Total	32	2	14	6	8	2

The differences shown here are small but probably important. Assuming that 70% of the added water will be retained in the bread, a decrease of 2% in absorption would mean a decrease of 2.8 lb. of bread per barrel of flour.

TABLE IV  
SUMMARY OF THE BAKING RESULTS

Number of cases in which	Baking formula						Total
	Simple	Malt*	Malt- phosphate**	Bromate	Blend- bromate	Malt- Arkady*	
<i>Loaf volume</i>							
Spring > fall	15	9	6	5	9	5	49
Spring = fall ± 2	13	7	4	12	8	4	48
Spring < fall	4	2	4	15	15	9	49
<i>Texture</i>							
Spring > fall	7	4	3	0	4	3	21
Spring = fall ± ½ point	21	9	9	23	16	11	89
Spring < fall	4	5	2	9	12	4	36
<i>Crumb color</i>							
Spring > fall	3	5	6	2	6	7	29
Spring = fall ± ½ point	26	8	7	19	10	7	77
Spring < fall	3	5	1	11	16	4	40
<i>Baking score</i>							
Spring > fall	15	8	7	7	6	6	49
Spring = fall ± 2	9	6	4	5	5	3	32
Spring < fall	8	4	3	20	21	9	65

\*1928 series only.

\*\*1929 series only.

Factors, other than absorption, which have been considered are loaf volume, texture, crumb color and appearance of the loaf. Of these, the loaf volume is the most important especially in testing experimentally milled flours for strength because, with the exception of absorption, the other characteristics of bread are subject to a wide range of modification by means of variations in the formula. It is true that loaf volumes will also vary with the formula but theoretically, at least, there should be a maximum loaf volume for each flour

that would depend only upon its inherent strength. The baking data have been summarized in Table IV. The formulas used fall into two classes, namely, those without and those with bromate. Table IV shows that in respect to volume and baking score, very little damage was apparent in the

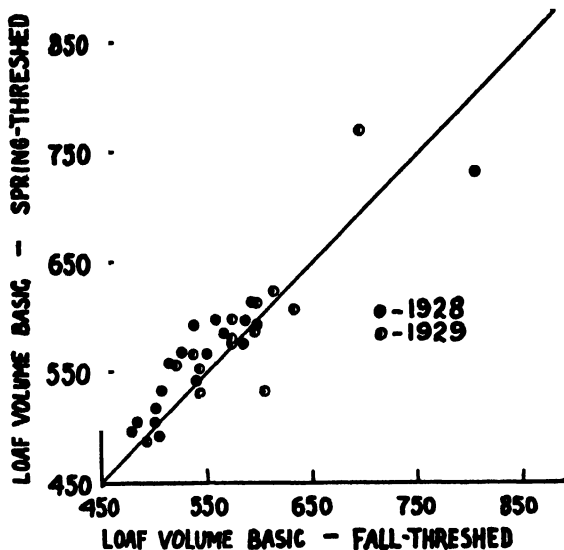


FIG. 2. Comparison of fall- and spring-threshed wheat in respect to loaf volume by the basic formula.

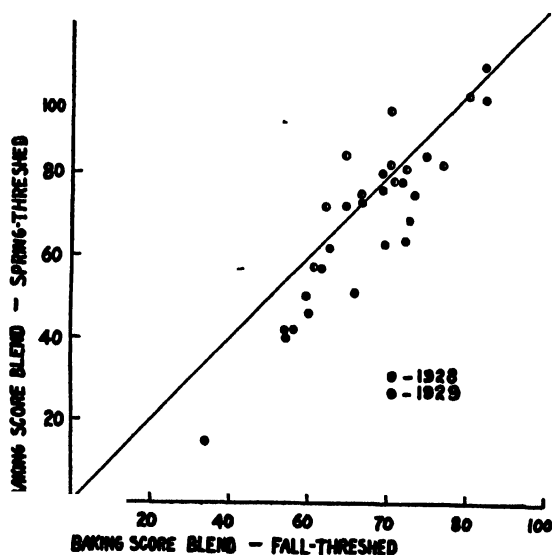


FIG. 3. Comparison of fall- and spring-threshed wheat in respect to baking score by the blend bromate formula.

spring-threshed samples when they were baked by formulas without bromate. On the contrary, the simple formula indicated that in most cases the quality had been somewhat improved in most of the samples. Twenty-eight of the thirty-two exposed samples were equal or better than their checks as regards loaf volume. This is shown more clearly in Fig. 2 where the loaf volumes of the exposed samples are plotted against the loaf volumes of their checks. This observation holds also for the malt and the malt-phosphate formulas, although in these cases the writers have only a single season's results.



It would be a mistake, however, to conclude from this that the winter exposure had resulted in an intrinsic improvement of quality, because on examination of the data obtained by the formulas including potassium bromate it is seen that in respect to both loaf volume and baking score, the exposed samples appear to be generally inferior to the fall samples. Thus by the blend-bromate formula 17 of the exposed samples were equal to or

TABLE V

## SUMMARY OF THE 1928 AND 1929 TESTS

Loaf volume of the spring-threshed as percentage of the corresponding fall-threshed sample

No.	Variety	of wheat	Simple	Bromate	Blend- bromate	Malt- phos- phate	Malt- Arkady	oxid- izers	oxid- izers	grade F to S
1928										
E 10		13.5	103	98	104	111	116	107	106	0
E 11		10.2	109	104	107	119	96	114	102	0
E 12		13.2	107	96	110	117	109	112	105	+1
E 13		10.6	111	102	96	136	104	124	101	-1
E 14		9.3	109	98	99	104	110	106	102	-1
E 15		10.8	104	87	95	102	97	103	93	-1
E 16		16.2	91	92	98	100	97	96	96	0
E 17		11.8	99	96	94	98	93	98	94	0
E 18		11.7	100	93	99	94	101	97	98	0
E 19		10.4	104	94	92	101	96	102	94	0
S 10	Marquis	13.0	98	94	92	92	106	84	102	-2
S 11	Red Fife	12.2	101	101	104	100	98	100	101	0
S 12	Red Fife	11.6	103	99	96	101	96	102	97	0
S 13	Marquis	10.6	104	102	95	102	96	103	98	-2
S 14	Marquis	12.7	101	104	101	104	108	102	104	-2
S 15	Red Fife	11.8	104	94	94	103	99	104	96	-1
S 16	Marquis	13.6	98	99	97	113	100	106	99	-1
S 17	Marquis	10.6	105	96	97	95	89	100	94	-2
Average								104	99	
1929										
E 50	Marquis	13.8	107	102	109	97		102	106	0
E 51	Garnet	15.2	102	90	102	103		102	96	0
E 52	Reward	12.7	100	89	105	93		96	97	0
E 53	Marquis	16.0	99	96	102	100		100	99	+1
E 54	Red Fife	15.4	101	99	101	97		99	100	0
E 55	Kitchener	14.7	101	120	100	97		99	110	+1
E 56	Garnet	15.8	101	99	104	111		106	102	-2
E 57	Marquis	14.5	110	102	103	105		108	102	-2
E 58	Renfrew	11.2	104	105	107	105		104	106	-1
W 1	Marquis	10.2	99	94	96	106		102	95	-1
W 2	Garnet	11.4	97	101	94	98		98	98	-1
W 3	Reward	14.5	96	106	97	100		98	102	0
W 4	Marquis	10.3	88	95	94	102		95	94	-2
W 5	Ceres	10.8	105	88	97	104		104	92	-2
Average								101	100	

better than their checks, while 15 were inferior, in respect to loaf volume, and in respect to baking score 21 of the 32 samples were inferior to the checks. This is shown graphically in Fig. 3 in which the blend-bromate baking scores of the exposed samples are plotted against the scores for the corresponding checks.

A more detailed treatment of the loaf volume results is given in Table V in which the samples of the two seasons are shown separately. The values are loaf volume of the spring-threshed as percentage of the loaf volume of the corresponding check sample. To facilitate comparisons, two means have been calculated for each sample, namely, the mean of the values obtained by the simple and malt-phosphate formulas, and the mean of the other formulas each of which included potassium bromate. By examining these two sets of means it should be possible to distinguish those samples which really were improved from those which were apparently improved but actually lowered in strength. If a sample showed improvement by both these sets of formulas, one would conclude that the improvement was real. When both sets show a decrease there can be little doubt that damage occurred. Considering only changes greater than 2% as significant, these results may be classified as in Table VI.

TABLE VI  
DISTRIBUTION OF SAMPLES WITH RESPECT TO LOAF VOLUME

Number of samples showing:	1928	1929	Total	%
Improvement	4	5	7	22
No change	6	6	12	38
Damage	8	5	13	40

It is apparent from these figures that on the whole more samples were damaged than were improved. In 7 of the 13 damaged samples the average decrease in loaf volume by the oxidizer formulas was in excess of 5% while in the 7 samples improved, 4 showed an average increase in excess of 5%.

### Conclusions

The effect of winter exposure on the quality of wheat may be summarized briefly in the following way:

The grade of the wheat was lowered in about 50% of the cases and the weight per bushel decreased in all but a very few instances. Flour yield was generally slightly increased but there occurred cases in which it was decreased.

The baking quality when judged by a formula including no oxidizer was usually improved but this appeared to be in many cases merely a kind of mellowing effect, because formulas with oxidizers in many cases showed decreased baking quality. In the series studied 38% of the samples exhibited no significant change, 40% evidence of damage, and 22% evidence of improvement as a result of the weathering they had received.

Of the 13 samples that showed evidence of damage due to weathering, 4 lost 2 grades, 3 lost 1 grade, and 6 were unchanged in grade. Altogether there were 8 samples that lost 2 grades. Of these 4 were damaged, 3 were unchanged and 1 was improved. There were 8 samples that lost 1 grade. Of these 3 were damaged, 4 were unchanged, and 1 was improved. Thirteen samples were unchanged in grade. Of these 6 were damaged, 5 were unchanged, and 2 were improved. The grade of the sample may be used as a rough criterion of change in baking quality, as in most cases where the grade is reduced, there is lowering of quality. It is not at all a dependable index of damage. It appears that the only safe criterion is a baking test of individual lots of wheat.

It should be pointed out, however, that the results brought out in this study very likely represent minimum effects. The outside storage of the samples took place in two seasons in which the autumn weather was quite dissimilar to the weather encountered in seasons when the farmers are forced to leave their wheat in the stook over winter. Under such circumstances the grain usually becomes thoroughly wet and is frequently subjected to a number of successive wettings and partial dryings before being finally frozen and snowed under. One might, therefore, reasonably expect to find that in a wet season the extent of damage by winter weathering would exceed the damage found in these two series.

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# THE EFFECT OF AGING AND HEAT ON THE CHROMOSOMAL MUTATION RATES IN MAIZE AND BARLEY<sup>1</sup>

BY F. H. PETO<sup>2</sup>

## Abstract

The recent discovery by Navashin (3), that the chromosomal mutation rate in *Crepis* was influenced by aging of the seed, has been corroborated by observations on the mutation rate of corn plants grown from seed of various ages.

A very high chromosomal mutation rate in barley was induced by heat treatments of seed under various conditions of humidity. The most common type of aberration resulting from these treatments appeared to be fracture of the chromosomes either at the attachment constriction, the secondary constriction or the point of attachment of the trabants. The reattachment of fragments to other chromosomes was observed in two instances.

Considerable importance is attached to the discovery that a large proportion of the mutant cells are eliminated during the growth of the plant. The principle that *only the fittest survive* seems equally true of cells as of individuals and groups of plants or animals.

## The Effect of Aging on the Mutation Rate

Navashin (3) reports that over 80% of the plants from seven-year-old seed of *Crepis tectorum* L. were chromosomal mutants of one sort or another while only 0.1% of the plants from fresh seed exhibited the same phenomenon.

The work outlined here was done in order to determine whether the above phenomenon was present in cereals grown from aged seed. Several inbred lines of maize were obtained from Professor L. C. Raymond of Macdonald College. Seed aged from one to six years of line II of this material was grown. In general there was a gradual decrease in germinability with the increase in age of the seed. There was a marked decrease in plant size and vigor in the plants from seed over a year old, and in addition a number of plants from seed lots over two years old were morphologically abnormal. Some were dwarfed and intensely green and died after reaching a height of a few inches; others were so heavily striped with chlorotic areas that they also died. A few of the more lightly striped plants survived but it is doubtful whether they will produce seed. A number of the chlorophyll-deficient plants resemble those produced when X-ray treatment was applied to corn by Stadler (5).

After the plants had been growing for two or three weeks root tips were obtained from 20 of each of six lots of seed of different ages. These were examined for chromosomal abnormalities after fixing with La Cour's 2BE fixative and staining with gentian violet. To date, results are available from three lots only. These are given in Table I and appear to corroborate Navashin's findings in *Crepis*. The remaining lines are being studied and any data collected will be presented in a later publication.

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TABLE I

EFFECT OF AGING ON THE MUTATION RATE IN MAIZE

Age of seed	Germination, %	Days from seeding to sampling of root tips	No. plants studied	Plants with mutated cells		No. cells observed	Mutated cells	
				No.	%		No.	%
6 months	92	22	20	0	0	120	0	0
5 years	76	32	19	3	16	112	5	4
6 years	32	33	20	5	25	155	13	8

Eighteen mutated cells were observed in plants from seed five and six years old. Seven of these cells contained 21 chromosomes, seven 19, one 18, two contained one fragment in addition to the normal number and one contained two complete sets of 20 chromosomes in the metaphase condition. These two nuclei were separated in the cytoplasm but within the same cell wall probably as a result of failure in wall formation after the previous somatic division. If this binucleate cell was also induced by aging it would indicate that all the mutants do not occur in the embryonic stage but throughout the life of the plant. The abnormal cells were located at random throughout the root tips, and it was impossible to find a large sector with all the cells exhibiting the same aberration. This indicates that most of the mutations were of relatively recent occurrence in the development of the root. Other mutations, however, may have occurred earlier in the embryonic and early seedling stage and subsequently have been eliminated. The reasons for suspecting this elimination will be considered later in this paper.

### The Effects of Heat on the Mutation Rate

Crocker and Groves (1, 2) investigated the effect of storage and heat on seeds, and from the relation between the time-temperature formula for the coagulation of proteins and the temperature-life-duration formula for seeds concluded that the loss of viability of the seeds was largely a matter of the coagulation of the cell proteins. Since it has been shown that loss of germinability in seeds is associated at least in its more advanced stages with an increase in the mutation rate, it is logical to suspect that any abnormal environmental factor that would favor the denaturation or coagulation of the nuclear proteins might be expected to result in an increased mutation rate. Consequently a series of experiments involving the treatment of O.A.C. No. 21 barley seeds under various conditions of temperature and moisture have been undertaken and the preliminary cytological findings are given in Table II.

TABLE II  
EFFECT OF HEAT ON THE MUTATION RATE IN BARLEY

Heat treatment	Germination	Material examined	No. plants studied	Plants with mutated cells		No. cells observed	Mutated cells	
				No.	%		No.	%
Untreated	99% in germinator	Root tips taken 30 days after seeding	12	0	0	41	0	0
95° C. for 25 min.	67% in germinator	Young seminal root tips	19	13	68	143	38	27
95° C. for 25 min.	67% in germinator, 32% in soil	Root tips taken 40 days after seeding	20	4	20	129	12	9
40° C. for 30 days	4% in germinator	Young seminal root tips	3	3	100	49	15	31

The barley was 1932 seed containing 9% moisture at the outset. In the treatment in a drying oven at 95° C. the seed was enclosed in a sealed glass capsule. There was a slight decrease in germinability after 15 min. heating. Further heating induced greater reduction in germination until at 35 min. the seed had lost its viability. There was a marked difference in the germination rate of seed placed in a Hearson's germinator and of that sown in soil. In soil the germination was lower owing probably to the less vigorous of the seedlings dying prior to emergence.

Of the seed treated at 95° C., only that exposed for 25 min. has been subjected to cytological examination. In one lot the seminal root tips were taken from the germinating seeds within a day or two after germination had commenced. In the other lot the root tips were taken from the plants 40 days after seeding. The difference in the mutation rate between these lots from seed treated in the same manner is very striking. There is a reduction in mutants from 68% in the seminal root tips to 20% in the plants 40 days after seeding. This indicates that there must be an elimination of the mutant cells during the development of the plant.

This is of great significance since it suggests that Darwin's principle of the survival of the fittest applies to cells as well as to individuals or groups of plants and animals. It suggests further that in plants grown under disadvantageous environmental conditions, there may occur a great profusion of mutant types, a large number of which for various reasons fail to survive for any length of time. The amount of initial variation which is induced is important, however, and while elimination of unfavorable mutants may take place earlier in the life of the plant than previously expected, any dominant or favorable mutants which might occur would undoubtedly have survival value.

No definite sectors carrying the same type of mutation could be found in the young seminal root tips. However, in the root tips from the older plants of the similarly treated seed, one large sector was found in which the same mutation occurred throughout, proving that this particular mutation had survived longer than those in the younger roots.

The chromosomal mutants observed in the barley heated at 95° C. consisted almost entirely of fragments. Approximately half of them were less than one-fifth of the average chromosome length. Some of these very probably had been trabants that had become disconnected from their chromosomes. The fractures resulting in the longer fragments appeared to have occurred in about equal numbers at the attachment constriction and secondary constrictions. In one instance a large fragment had become reattached to a normal chromosome.

According to Navashin's (4) dislocation hypothesis regarding the evolution of chromosome numbers, a fracture cannot occur at the exact point where the kinetic constriction (attachment constriction) is located. Nevertheless preliminary studies of the heat treated material indicates that fractures do occur quite frequently at this point. Further studies should supply more definite information.

Preliminary results are also given on barley seed treated at 40° C. in an open dish in an unventilated incubator. A large pan of water was placed in the bottom of the incubator so that the air must have remained close to the saturation point. The incubator was opened daily to prevent the air from becoming induly stagnant. The percentage germination was reduced after five days treatment, and after 30 days treatment only 4% of the seeds remained viable. It was from three of these germinating seeds that the seminal root tips were taken. This was the most severe treatment applied and as would be expected the effects on the chromosomes were most pronounced. In addition to the types of fragmentation observed in the barley, whole chromosomes were occasionally missing, one cell contained only 11 chromosomes and two very small fragments.

These results show that the effect of heat on the chromosomal mutation rate is equally striking at low and high temperatures, providing adequate time is allowed for the treatment at the lower temperatures. This leads one to suspect that certain adverse environmental conditions in nature may be as effective in inducing changes in the chromosomal constitution of plants as is aging under natural conditions.

The evidence presented would appear to strengthen the mutationist's concept of evolution, but it indicates that the environment may play a large part in influencing the rate of mutation.

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# THE MARINE ALGAE OF THE MARITIME PROVINCES OF CANADA

## I. List of Species with their Distribution and Prevalence<sup>1</sup>

BY HUGH P. BELL<sup>2</sup> AND CONSTANCE MACFARLANE<sup>3</sup>

### Abstract

The marine algae of the Atlantic coast of the maritime provinces of Canada were collected at representative places all along the coast. The most intensive collecting was done at St. Andrews, New Brunswick, and at Halifax, Nova Scotia. An entire summer was spent collecting around Prince Edward Island. The report covers the work of more than seven years. The collecting was done chiefly during the summer, but regular collecting was also carried out for two winters. The area is divided into three distinct geographical and ecological regions, namely, the Bay of Fundy, the Atlantic, and the Prince Edward Island regions. In the list of species, their regional distribution and prevalence are given in tabular form. The list includes 120 species, divided into 30 Chlorophyceae, 41 Phaeophyceae, and 49 Rhodophyceae. In addition to critical notes regarding certain forms, the striking differences in the marine flora from region to region are indicated diagrammatically by distribution maps for a number of species.

The following article is a statement regarding the occurrence, prevalence, and distribution of the more common forms of marine algae found along the shores of the maritime provinces. Workers in marine biology have often been in need of information of a general character regarding the distribution of the more common marine algae of this region, and as there is no up-to-date manual covering the forms found on the Canadian Atlantic coast, such information is difficult to obtain. This account is an attempt to provide the required information in an easily accessible form.

The original intention was to base this report on the results of intensive collecting at a limited number of points. However during the summer of 1927, Dr. Huntsman, of the Biological Board of Canada, instructed his workers throughout the maritime provinces to send in collections of seaweeds for identification in the Botanical Laboratories of Dalhousie University. From an examination of these collections it was at once apparent that there was great variation in the marine flora throughout the area and that it would therefore be necessary to carry out a more extensive program, including collecting all along the coast. It was not until the summer of 1931 that this program was completed. As a result the survey has extended over quite a number of years. Starting in 1924, two summers were spent collecting algae at St. Andrews, New Brunswick, four summers at the same work in Halifax, and one summer collecting at various points around Prince Edward Island. During the summers spent at St. Andrews and Halifax, numerous preliminary

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trips were made to other places, and in 1931 the coast of the maritime provinces was covered in a systematic manner. This was accomplished by numerous and extensive trips in a small car, and as a result collections were made at points all along the coast from Gaspé, Quebec, to Grand Manan, New Brunswick, inclusive. Most of the collecting stations are indicated on Map 2. To cover this ground and to visit the important stations a sufficient number of times necessitated over 8,000 miles of travel during the summer of 1931 alone. Although collections were taken on the shores surrounding the Gaspé Peninsula, this report does not cover that region, for the marine flora of that district is quite different from the flora of the New Brunswick and Prince Edward Island coasts. Bay Chaleur must therefore be regarded as the northern limit for the information given in this report. The possibility arose that a number of forms might occur only during the winter months. Such plants would have been missed in the summer collecting. In order to overcome this difficulty, regular and frequent observations were made at the mouth of Halifax Harbor during two winters. In spite of thorough search no winter forms were found which had not already been seen either early or late in the summer. Thus for the purpose of a preliminary survey of the common forms, the coast of the maritime provinces has been well covered.

In addition to the coastal regions, the warm Bras d'Or Lakes in the centre of Cape Breton were also investigated. The species there, though less plentiful than on the Atlantic coast, conform in a general way to those found in the latter region. But in the lakes the condition of the algae is so atypical and the ecological conditions so unlike those of the ocean that their discussion does not properly belong here and is left to a later paper.

For ease of reference the species listed are divided into the classes, Chlorophyceae (Greens), Phaeophyceae (Browns), and Rhodophyceae (Reds). In each class the species are tabulated alphabetically according to genera. The coast is divided by nature into three distinct geographical and ecological regions; namely, (1) the coast bordering the Bay of Fundy, (2) the eastern Atlantic coast of Nova Scotia, and (3) the coast of Prince Edward Island together with the coasts of New Brunswick, Nova Scotia, and Cape Breton, bordering on the Northumberland Strait and the Gulf of St. Lawrence. For convenience these are called the "B. of F. Region," the "Atlantic Region," and the "P.E.I. Region." The regional prevalence of each species is indicated by the self-explanatory terms, "abundant," "common" and "occasional." Their use in each case is not based on any actual counts. They are used merely to represent the general impression obtained regarding the comparative prevalence of the various species. When there was no record obtained for a species in one of the regions during this survey, the absence of such record is indicated by a blank. A blank space does not necessarily mean that the species is absent from the region, but it indicates at least that it is not very common there. It is desirable to record certain detailed information regarding some of the species. This is given in the form of notes immediately following the tables.

It should be explained that for some species the terms used to denote prevalence are not entirely satisfactory and in certain instances may not convey a correct idea. For during the course of this work one was much impressed by the rapid change which may occur in some species of marine flora. Plants of a certain species may be abundant in a given region for the greater part of a summer and not be seen there again for a long time. Or again, plants of some species may appear in great abundance for a few days only, but not be seen at all either before or after. In these individual cases, if observations had been made during a different year or at another time of the same year, a different term might have been selected to indicate the prevalence. But most species are not so variable or so perishable as this, and cases where observations suggest that a crop is of short duration are dealt with in the notes.

The dominant forms in the marine flora changed so abruptly from region to region that it was considered advisable to supplement the species list by distribution maps of a few species selected to illustrate typical differences. The occurrence of a species along any part of the coast is indicated by dots. The prevalence of a species is indicated by the dots being either close together, slightly separated, or quite far apart, indicating that the species is "abundant," "common," or "occasional." It will be noted from these maps that the prevalence of a species is fairly uniform throughout each region, but may change abruptly in going from one region to another.

When working with the marine algae of the Northwestern Atlantic, the works which one usually consults are those of Harvey (7, 8), Farlow (6), Collins (1, 2, 3, 4), Eaton (5) and Klugh (9). There is also a recent report for the extreme north of Cape Breton (St. Paul's Island) by Roscoe (11). In making identifications these works have been consulted continually. However, each of the authors mentioned above reports species for this region which are not mentioned in this article, for the present report is merely a record of observations made during this survey. Whenever possible the specific names used are taken from the list of marine algae published by Collins (1, 2, 3, 4). Those not included in Collins' list are made to conform to De Toni (13). Biological workers in the maritime provinces not familiar with the algae of the region would find a useful guide in "A Handbook of the British Seaweeds" by Lily Newton (10). This recently published handbook contains good keys and general descriptions, and it is well and copiously illustrated. Practically all the species found in the area considered here are described in this manual.

There are no Blue-greens or other microscopic forms included in this list, because this article deals only with conspicuous forms regarding which the average worker would require information, and the Blue-greens, etc., do not come under that head. There are however many species of Blue-greens to be found along the shores of the maritime provinces. Some of them form quite extensive gelatinous coatings over the surfaces of the rocks. Many others live as parasites or epiphytes on other algae. These blue-green plants are nearly all microscopic, and at present they are not likely to play a conspicuous part in non-botanical collections. They are therefore not included in the present report.

As far as could be ascertained from the records available the list that follows includes only two species which are reported for this area for the first time. These are *Monostroma arcticum*, Wittr. and *Stypocaulon scoparium*, (L.) Kuetz.

TABLE I  
SPECIES LIST

Species	Bay of Fundy	Atlantic	Prince Edward Island	Map	Notes
<b>CHLOROPHYCEAE</b>					
<i>Bryopsis plumosa</i> , Ag.	—	Occasional	—		
<i>Chaetomorpha aerea</i> , (Dillw.) Kuetz.	—	—	Occasional		
<i>Chaetomorpha aerea</i> , (Dillw.) Kuetz. forma <i>Linum</i> , (Muell.) Collins	—	Common	—		
<i>Chaetomorpha melagonium</i> , (Web. & Mohr.) Kuetz., forma <i>rupicola</i> , Aresch.	Occasional	—	Occasional		
<i>Chaetomorpha melagonium</i> , (Web. & Mohr.) Kuetz., forma <i>typica</i> , Kjellm.	—	Common	—		
<i>Cladophora crystallina</i> , Kuetz.	—	Occasional	—		
<i>Cladophora flexuosa</i> , (Griff.) Harv.	—	Common	Common		
<i>Cladophora flexuosa</i> , (Griff.) Harv., forma <i>densa</i> , Collins	—	Occasional	—		
<i>Cladophora gracilis</i> , (Griff.) Kuetz.	—	Common	—		
<i>Cladophora hirta</i> , Kuetz.	—	Occasional	—		
<i>Cladophora rupestris</i> , (L.) Kuetz.	Occasional	—	—		
<i>Cladophora virgatula</i> , Grunow	—	—	Occasional		2.
<i>Codium pusillum</i> , (Lyngb.) Kjellm.	—	Occasional	—		
<i>Enteromorpha compressa</i> , (L.) Grev.	Abundant	Abundant	Common		
<i>Enteromorpha intestinalis</i> , (L.) Link, forma <i>clavata</i> , J. Ag.	Abundant	Abundant	Abundant		
<i>Enteromorpha intestinalis</i> , (L.) Link, forma <i>cylindracea</i> , J. Ag.	Common	Common	Common		
<i>Enteromorpha linza</i> (L.) J. Ag.	Abundant	Abundant	Abundant		
<i>Enteromorpha minima</i> , Naeg., forma <i>glacialis</i> , Kjellm.	Common	Common	Common		
<i>Enteromorpha percursea</i> , (Ag.) J. Ag.	Occasional	—	—		
<i>Enteromorpha prolifera</i> , (Muell.) J. Ag.	Common	Common	Common		
<i>Hormiscia penicilliformis</i> , (Roth) Fries	Common	Common	—		
<i>Monostroma arcticum</i> , Wittr.	Occasional	—	—		
<i>Monostroma fuscum</i> , (Post. & Rupr.) Wittr.	Abundant	Abundant	Common		
<i>Rhizoclonium riparium</i> , (Roth) Harv., variety <i>implexum</i> , Rosen.	Occasional	—	—		
<i>Rhizoclonium riparium</i> , (Roth) Harv., variety <i>polyrhizum</i> , Rosen.	Common	—	—		
<i>Rhizoclonium tortuosum</i> , Kuetz.	Common	Common	—		
<i>Rhizoclonium tortuosum</i> , Kuetz., forma <i>polyrhizum</i> , Holden.	Occasional	Occasional	—		
<i>Spongomorpha arcta</i> , (Dillw.) Kuetz.	Abundant	Abundant	Occasional	3.	
<i>Spongomorpha spinescens</i> , Kuetz.	—	Occasional	—		3.
<i>Ulva lactuca</i> , L., variety <i>rigida</i> , (Ag.) Le Jolis	—	Occasional	Abundant		4.
<b>PHAEOPHYCEAE</b>					
<i>Agarum turneri</i> , Post. & Rupr.	Abundant	Abundant	Common		
<i>Alaria esculenta</i> , (L.) Grev.	Abundant	Abundant	—	4.	
<i>Ascophyllum machaili</i> , (Turn.) Holmes & Batters	—	Occasional	—		
<i>Ascophyllum nodosum</i> , (L.) Le Jolis	Abundant	Abundant	Occasional	3.	
<i>Asperococcus echinatus</i> , (Mert.) Grev.	—	Common	—		5.
<i>Castagnea virescens</i> , (Carm.) Thuret	—	Occasional	—		6.
<i>Castagnea zosterae</i> , (Mohr.) Thuret	—	Common	—		
<i>Chaetopteria plumosa</i> , (Lyngb.) Kuetz.	—	—	Common		

TABLE I—Continued

## SPECIES LIST

Species	Bay of Fundy	Atlantic	Prince Edward Island	Map	Notes
<b>PHAEOPHYCEAE—Concluded</b>					
<i>Chorda filum</i> (L.) Stackh	Abundant	Abundant	Common		7.
<i>Chordaria flagelliformis</i> (Muell.) J. Ag	Abundant	Abundant	Common		
<i>Dermarestia aculeata</i> (L.) Lamour	Common	Common	Common		8.
<i>Dermarestia viridis</i> (Muell.) Lamour	Common	Common	Occasional		
<i>Dermotrachelum undulatum</i> (J. Ag.) Renke	—	Occasional	Abundant		
<i>Dictyosiphon foeniculaceus</i> (Huds.) Grev	Common	Common	Abundant		
<i>Ectocarpus confervoides</i> (Roth) Le Jolis	Abundant	Abundant	Abundant		9
<i>Ectocarpus tomentosus</i> (Huds.) Lyngb	—	Occasional	—		9 & 10
<i>Flachisteia fuscicola</i> (Vell.) Fries	Common	Common	—		
<i>Fucus evanescens</i> Ag	Common	Abundant	Common		11
<i>Fucus platycarpus</i> Thuret	Common	Abundant	Occasional		
<i>Fucus serratus</i> L.	—	Occasional	Abundant	5	
<i>Fucus vesiculosus</i> L.	Abundant	Abundant	Abundant		11
<i>Fucus vesiculosus</i> L. variety <i>sphaerocarpus</i> Farlow	Occasional	Occasional	—		12
<i>Ilea fasciata</i> (Muell.) Fries	Abundant	Abundant	Abundant		13
<i>Ilea zosteraefolia</i> (Reinke) S. & G.	Occasional	Occasional	—		
<i>Laminaria agardhii</i> Kjellm	Abundant	Abundant	Abundant		14
<i>Laminaria digitata</i> Lamour	Abundant	Abundant	Common		
<i>Laminaria longicruris</i> De la Pyl	Abundant	Common	—	6	15
<i>Laminaria phyllitis</i> (Stackh.) Lamour	—	—	Abundant	7	
<i>Mesogloia divaricata</i> (Ag.) Kuetz	Common	Common	Common		
<i>Mesogloia vermicularis</i> Ag	—	—	Occasional		
<i>Punctaria latifolia</i> Grev	—	Abundant	Abundant		16
<i>Punctaria plantaginea</i> (Roth) Grev	—	Occasional	—		
<i>Pyralisella littoralis</i> (L.) Kjellm	Abundant	Abundant	Abundant		
<i>Ralfsia verrucosa</i> Areach	Common	Common	Common		
<i>Saccorhiza dermatodea</i> (De la Pyl.) J. Ag	Abundant	Common	Occasional	8	
<i>Sargassum</i> sp.	—	Occasional	—		17.
<i>Scytosiphon lomentarius</i> (Lyngb.) J. Ag	Abundant	Abundant	Common		
<i>Sphacelaria curvirostris</i> (Roth) Ag	—	—	Occasional		
<i>Sphacelaria radicans</i> (Dillw.) Ag	Common	—	—		18
<i>Stilophora rhinodes</i> (Ehrh.) J. Ag	—	—	Common		19.
<i>Stypocaulon scoparium</i> (L.) Kuetz	—	—	Abundant	7	

## RHODOPHYCEAE

<i>Actinococcus subcylindricus</i> (Lyngb.) Rosenv	—	—	Abundant	7.	20.
<i>Aknefeltia phycata</i> (Huds.) Fries	Common	Common	Common		
<i>Anethammon cruciatum</i> , (Ag.) Naeg	—	Occasional	Common		
<i>Anethammon floccosum</i> (Muell.) Kleen	—	Common	Occasional		
<i>Bangia fusco-purpurea</i> , (Dillw.) Lyngb	Common	Common	—		
<i>Callithamnion baileyi</i> Harv	—	Occasional	Occasional		
<i>Callithamnion corymbosum</i> , (Sm.) Lyngb	—	Occasional	—		
<i>Callithamnion roseum</i> (Roth) Harv	—	Occasional	Occasional		
<i>Ceramium circinnatum</i> , (Kuetz.) J. Ag	—	—	Occasional		21.
<i>Ceramium diaphanum</i> , (Lightf.) Roth	—	—	Occasional		21
<i>Ceramium elegans</i> , Ducl	Common	—	—		21 & 22.
<i>Ceramium rubrum</i> (Huds.) Ag	Occasional	Common	Common		21.
<i>Ceramium rubrum</i> , (Huds.) Ag, variety <i>decurrens</i> (Kuetz.) Harv	—	—	Common		21.
<i>Ceramium strictum</i> , Grev & Harv	—	Occasional	Common		21 & 23.
<i>Chondrus crispus</i> , (L.) Stackh	Occasional	Common	Abundant	9.	
<i>Corallina officinalis</i> , L.	Common	Common	Common		
<i>Cystodinium purpurascens</i> , (Huds.) Kuetz.	Abundant	Common	Common		
<i>Delesseria alata</i> , Lamour	—	Occasional	—		24.

TABLE 1—Concluded

## SPECIES LIST

Species	Bay of Fundy	Atlantic	Prince Edward Island	Map	Notes
<b>RHODOPHYCEAE—Concluded</b>					
<i>Delesseria sinuosa</i> , (Good. & Woodw.) Lamour	Common	Common	Common		
<i>Dumontia filiformis</i> , (Muell.) Grev.	Common	Common	—		
<i>Euthora cristata</i> , (L.) J. Ag.	Common	—	Occasional		25.
<i>Furcellaria fastigiata</i> , (Huds.) Lamour	—	—	Abundant	7.	
<i>Gigartina mamillosa</i> , (Good. & Woodw.) J. Ag.	Abundant	Common	—	6.	26.
<i>Gloiosiphonia capillaris</i> , (Huds.) Carm.	—	Occasional	—		27.
<i>Gracilaria confervoides</i> , (L.) Grev.	—	—	Abundant		28.
<i>Halosaccion ramentaceum</i> , (L.) J. Ag.	Common	Abundant	—	10.	
<i>Halosaccion ramentaceum</i> , (L.) J. Ag., variety <i>gladiatum</i> , Eaton	Common	Occasional	—		29.
<i>Hildenbrandtia rosea</i> , Kuetz.	—	Occasional	—		
<i>Lithothamnion</i> spp.	Abundant	Abundant	Abundant		30.
<i>Melobesia le jolisii</i> , Rosan.	Common	Common	Common		
<i>Nemalion multifidum</i> , (W. & M.) J. Ag.	—	Occasional	—		
<i>Petrocelis cruenta</i> , J. Ag.	—	Occasional	—		
<i>Phyllophora brodiaei</i> , (Turn.) J. Ag.	—	—	Abundant	7.	
<i>Phyllophora membranifolia</i> , (Good. & Woodw.) J. Ag.	Occasional	—	Abundant		
<i>Plumaria elegans</i> , (Bonnem.) Schmitz	Occasional	—	Occasional		
<i>Polyides rotundus</i> , (Gmel.) Grev.	—	Occasional	—		31.
<i>Polysiphonia elongata</i> , (Huds.) Harv.	—	—	Common		
<i>Polysiphonia fastigiata</i> , (Roth) Grev.	Common	Common	—		
<i>Polysiphonia harveyi</i> , Bailey	—	—	Occasional		32.
<i>Polysiphonia nigrescens</i> , (Dillw.) Grev.	—	Common	Common		
<i>Polysiphonia olneyi</i> , Harv.	—	Occasional	Occasional		32.
<i>Polysiphonia spinulosa</i> , Grev.	—	—	Occasional		33.
<i>Polysiphonia urceolata</i> , (Lightf.) Grev.	Abundant	Common	Abundant		
<i>Polysiphonia violacea</i> , (Roth) Grev.	—	Abundant	Common		
<i>Porphyra laciniata</i> , (Lightf.) Ag.	Abundant	Abundant	Abundant		
<i>Ptilota pectinata</i> , (Gunn.) Kjellm.	Common	Common	Common		
<i>Rhodochorton rothii</i> , (Turt.) Naeg.	Common	Occasional	—		
<i>Rhodomela subfusca</i> , Ag.	Occasional	Abundant	Abundant		
<i>Rhodymenia palmata</i> , (L.) Grev.	Abundant	Common	Common		

## Notes

1. *Bryopsis plumosa*. Found only in the warm waters of the Thrumcap lagoon at the mouth of Halifax Harbor.

2. *Cladophora virgatula*. When seen in the water this form was distinctly different from any other *Cladophora* of the region, and it was found a number of times at various places around Prince Edward Island. It conforms to the description of *C. virgatula* given by Collins, but, as he reports it from the West Indies only, there is naturally some doubt as to its being this species.

3. *Spongomorpha spinescens*. Found only at New Harbor on the north-eastern side of Mahone Bay, N.S.

4. *Ulva lactuca* var. *rigida*. Other reports give this form as being common for this entire area, but during the present survey although diligent search

was made for it, no plants have been seen in the Bay of Fundy, and but few on the Atlantic coast. It was not found in any exposed situation but it was very plentiful in the P.E.I. region at the heads of warm bays and estuaries.

5. *Asperococcus echinatus*. This form may occur in the other regions also. It is possible that in the B. of F. and the P.E.I. regions it may have been unnoticed among the *Scytosiphon lomentarius* of which there was a great quantity.

6. *Castagnea virescens*. This occurred in great profusion for a short period during one summer in the Thrumcap lagoon, Halifax Harbor, but it has not been observed in abundance elsewhere, and repeated search has failed to locate it again at Thrumcap.

7. *Chorda filum*. Most manuals on algae do not lay sufficient emphasis on the striking change which takes place in this plant during the spring and summer. In the early spring it is profusely coated with dark brown hairs, so that it appears to be encased in a coat of dark heavy fur. Towards summer, these hairs become transparent and begin to drop off, until in the late summer the plant is completely denuded and becomes thin and stringy in appearance.

8. *Desmarestia aculeata*. When attached this plant is covered with a thick coating of dark brown hairs. When washed up on the beach the hairs are lacking for they disappear very quickly when the plant is removed from its sublittoral habitat. The change in appearance is so great that it is sometimes difficult to realize that the black wire-like plant found on the beach is the same species as the brown velvety plant seen waving in the water below the low tide mark.

9. *Ectocarpus*. There are undoubtedly several other species throughout this area, but due to the abundance of *Ectocarpus*, the similarities in appearance of various species in the field, and the large area covered during the survey, it was not possible to make a thorough investigation of species of this genus.

10. *Ectocarpus tomentosus*. Identified only from Clam Bay, N.S.

11. *Fucus evanescens* and *F. vesiculosus*. In the P.E.I. region the plants of these two species are very small.

12. *Fucus vesiculosus* var. *sphaerocarpus*. Due to the small size of the type in P.E.I. the variety may have been overlooked in this region.

13. *Ilea fascia*. Branching plants of this species were observed toward the seaward end of the breakwater at Borden, P.E.I.

14. *Laminaria agardhii*. The various manuals or lists referring to the marine algae of this area include *Laminaria saccharina* as occurring here. But during the present survey, every type of *Laminaria* found has been carefully sectioned through both lamina and stipe, and no mucilaginous ducts could be seen. The common *Laminaria* of these waters was therefore identified as *L. agardhii*.

15. *Laminaria longicuris*. This alga is of more frequent occurrence than would appear from the amount found washed up on the shores after storms.

This is largely due to its habitat in deep quiet waters. By dredging in such places as the Northwest Arm, Halifax, N.S., the plants can be found in large quantities.

16. *Punctaria latifolia*. This form is frequently found in great abundance, but is spasmodic in occurrence. For a brief period during the summer farmers can be seen taking it up in cartloads to be used as fertilizer, but for the rest of the year it is entirely lacking in that locality.

17. *Sargassum*. Specimens of *Sargassum* sp. were brought in by Dr. A. W. H. Needler, from Lockport, N.S. He reported, "There was quite a bit of this on the beach at Lockport on October 30, enough I would say to make it easy for one to get several bushels of the fresh weed in half an hour. The next day it had almost entirely disappeared, its place being taken by *Fucus* . . . ." This is the only time *Sargassum* was observed in this region, and due to the dried state of the plants when received it was impossible to distinguish the species.

18. *Sphacelaria radicans*. Collected only at the mouth of the Bay of Fundy.

19. *Stilophora rhizodes*. This was found in great quantities in warm landlocked tidal estuaries on the north shore of Prince Edward Island, especially at Bayview. It probably occurs in similar habitats elsewhere.

20. *Actinococcus subcutaneus*. Doubt has been cast on the validity of this species. Rosenvinge (12) declares it to be an asexual generation of *Phyllophora brodiaei* growing parasitically on the sexual generation. Similarly, concerning another species of *Actinococcus*, a note by Newton (10, p. 413), states in part, "Miss B. D. Gregory, permits me to quote her unpublished work, which has established the fact that the pustule, formerly referred to the genus *Actinococcus* Kutz., and believed to be parasitic on *Gymnogongrus griffithsiae* Mart., is actually the tetrasporic nemathecium of the *Gymnogongrus*."

21. *Ceramium*. The classification of the species of this genus is in an unsatisfactory state at the present time, hence there is some doubt as to the nomenclature of the species here named, except in the case of the most common forms.

22. *Ceramium elegans*. Collected only at the mouth of the Bay of Fundy. It occurs in exposed situations only and grows in clumps that hang from rocky ledges. It is usually covered by other algae.

23. *Ceramium strictum*. Found only in very warm waters such as Souris beach, estuaries at Malpeque, Bayview, Lot 40, etc., P.E.I., and at Caribou Harbor near Pictou, N.S.

24. *Delesseria alata*. Only one specimen of this was found. It was obtained by dredging in Halifax Harbor.

25. *Euthora cristata*. Found at the mouth of the Bay of Fundy and on the north shore of Prince Edward Island. When observed it was in great abundance, but it occurred at infrequent intervals.

26. *Gigartina mamillosa*. On the Atlantic coast this form is local in distribution. Around the Bay of Fundy shores it is of more general distribution, and in places such as Sandy Cove near Digby it forms a thick mat over the flat surfaces of the rocks.

27. *Gloiosiphonia capillaris*. This is a transient form occurring for a few weeks in the summer, and has been found at only one spot near the mouth of Halifax Harbor. It has, however, been collected at the same time during two consecutive summers, and though a transient it would therefore seem to be a regular form for Halifax.

28. *Gracilaria*. *G. confervoides* has been found associated with *Stilophora rhizoides* in the warm waters of the landlocked tidal estuaries of P.E.I. The plants are heavy in structure, pale in color, and grow in bushy clumps. A part of a plant of *Gracilaria* sp. was also found washed upon the beach at the mouth of Halifax Harbor.

29. *Halosaccion ramentaceum* var. *gladiatum*. In the early spring the *Halosaccion* shows the typical wire-like cylindrical structure and compressed cartilaginous texture. A few weeks later in certain regions some plants can be seen to become gradually inflated or broad and flat. At the same time they become much softer and grow quite long (from about 8 in. winter length to 18 in. in spring). It is therefore doubtful whether this is a true variety, or merely a summer condition for certain regions.

30. *Lithothamnion*. No attempt was made to identify the various species of *Lithothamnion* of which there are a great number in this area.

31. *Polyides rotundus*. The identification of this species is uncertain. No fruiting plants have been found.

32. *Polysiphonia harveyi*, and *P. olneyi*. Much doubt exists concerning these two species. They are all gradations from the slender *P. olneyi* to the setaceous *P. harveyi*.

33. *Polysiphonia spinulosa*. Only two small pieces of this were collected, and though searched for carefully, not enough could be found for complete identification.

### Explanation of Maps

#### MAP 1.

Maritime provinces with the exception of northern New Brunswick, showing the area covered during the survey and the main regions to which reference is made in this article.

#### MAP 2.

Area covered by survey showing the principal collecting stations.

#### MAPS 3-10.

These maps indicate the sharp change in marine flora from region to region. The presence of a species is indicated by dots along the part of the shore where it was collected. Their abundance is indicated by the relative distance between dots. In every case the species having the indicated distribution are listed on the map, and the map numbers are given in the species list.



### Acknowledgments

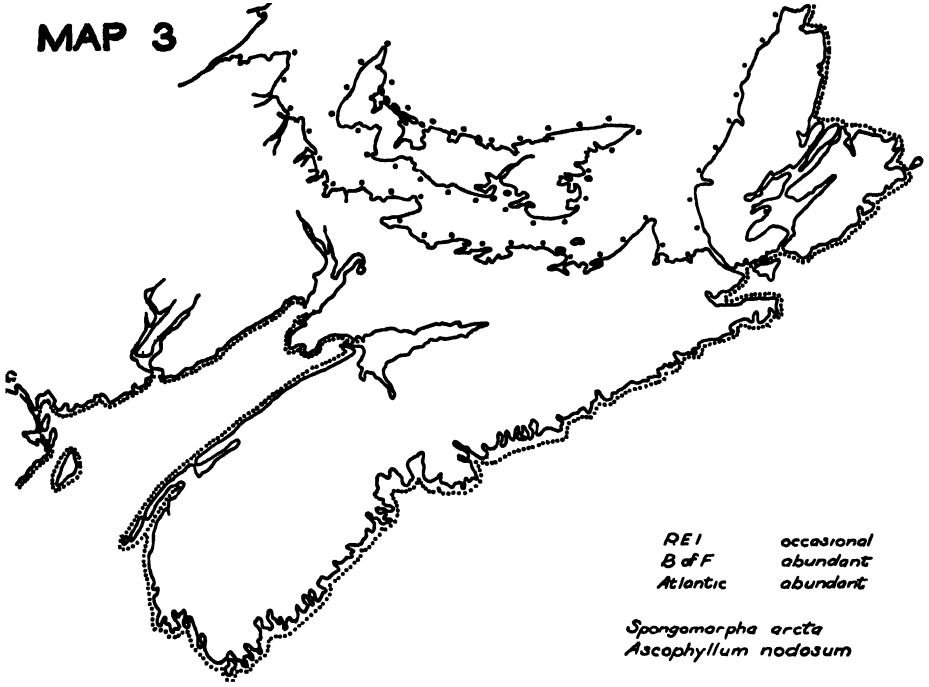
Thanks are due especially to Dr. W. R. Taylor of the University of Michigan for his advice and assistance so freely given. Without his thorough knowledge and expert guidance many species could not have been identified with certainty, and the report thus brought to a satisfactory conclusion.

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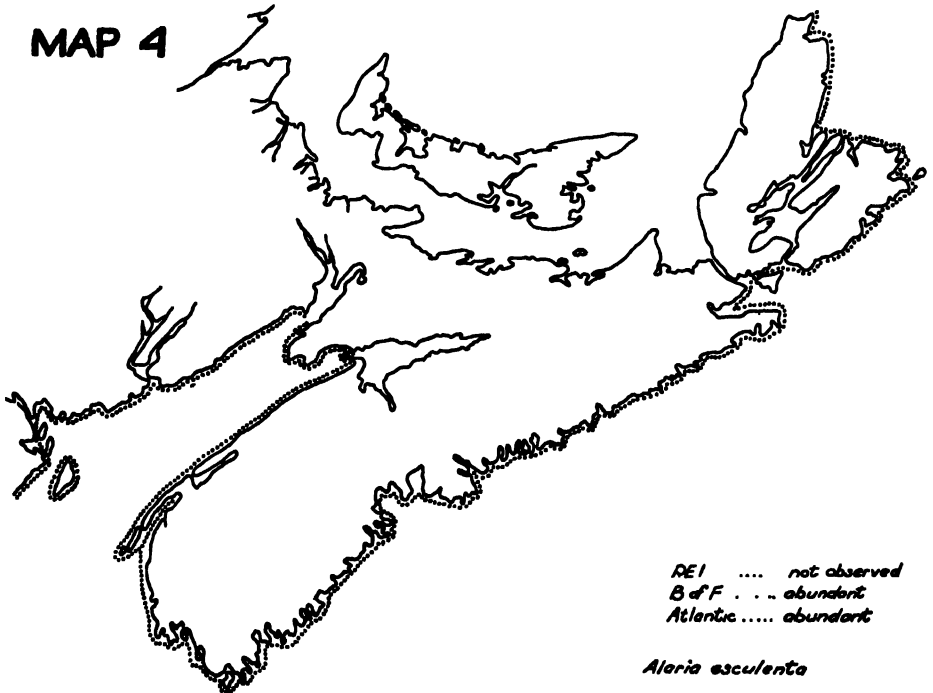
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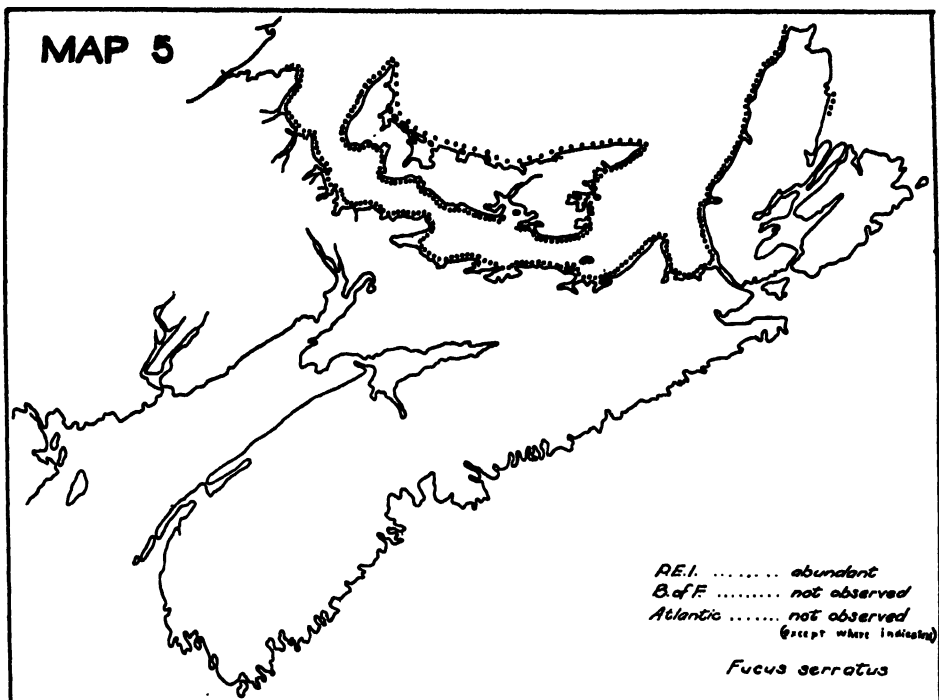
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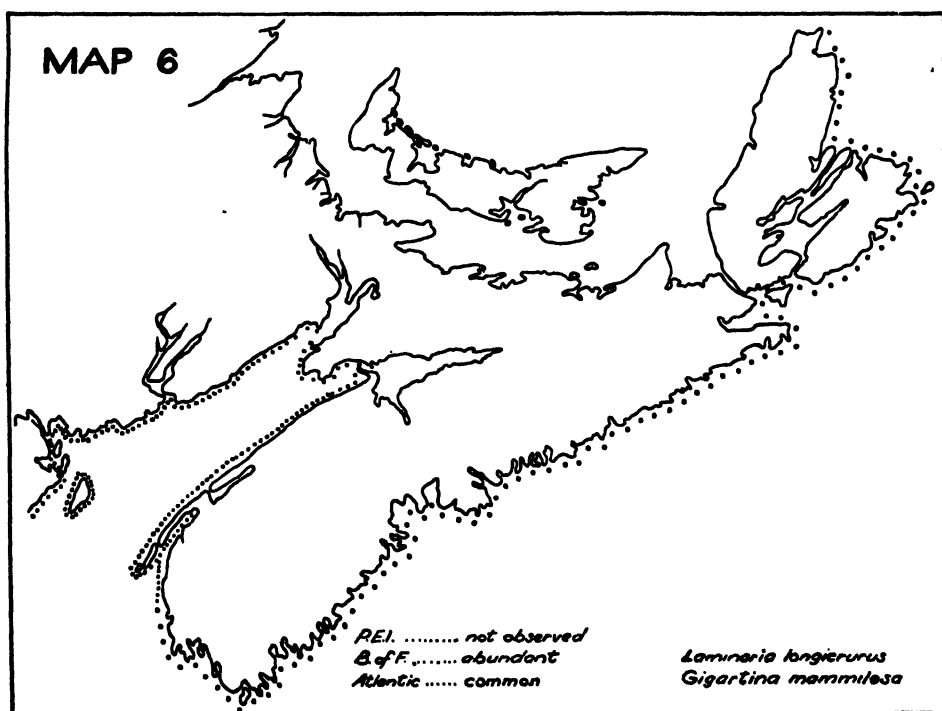
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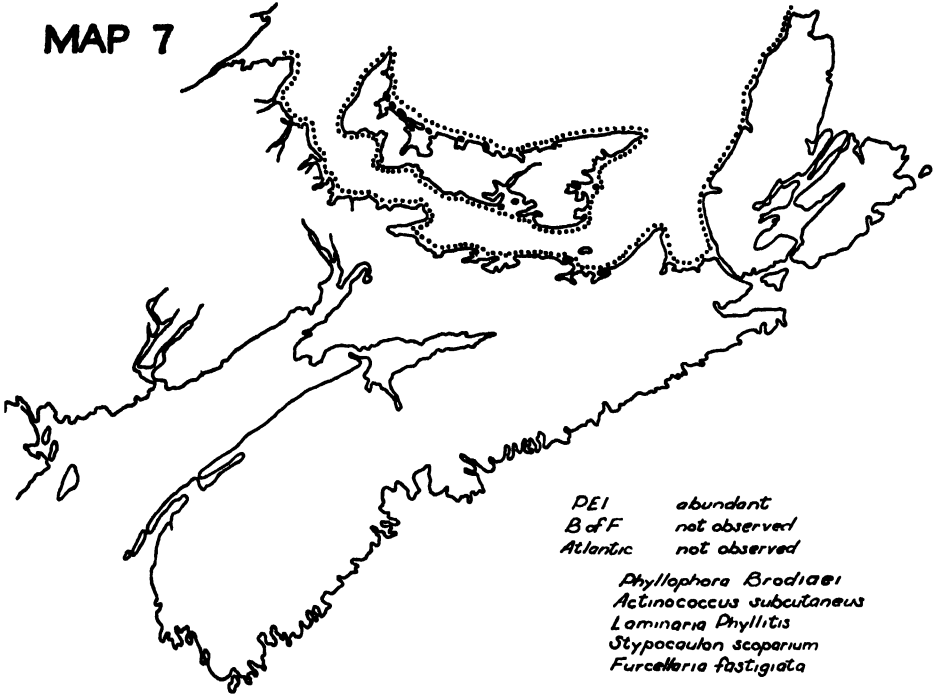
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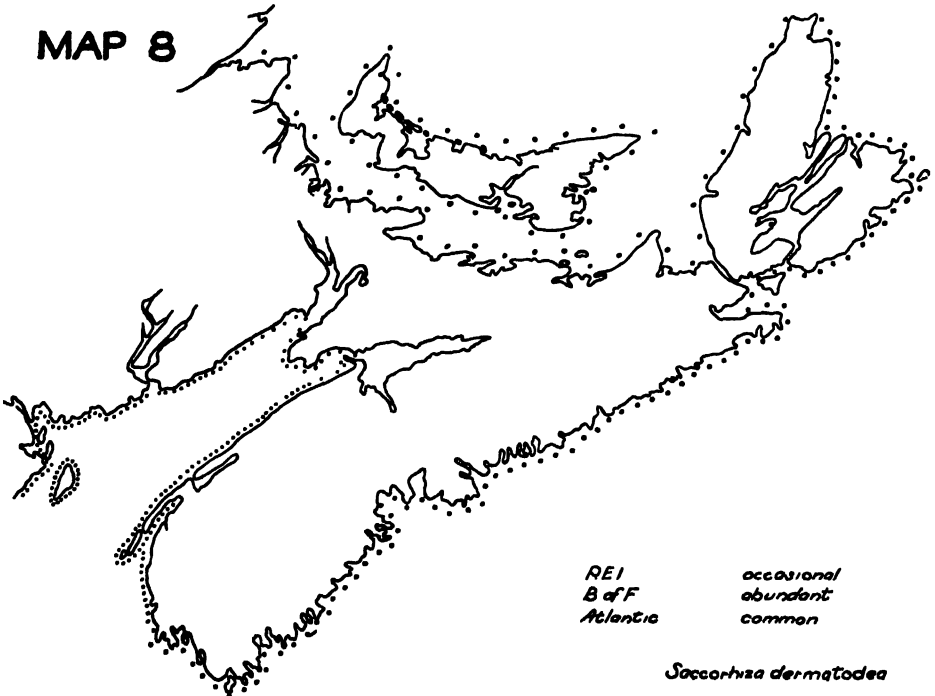
MAP 6



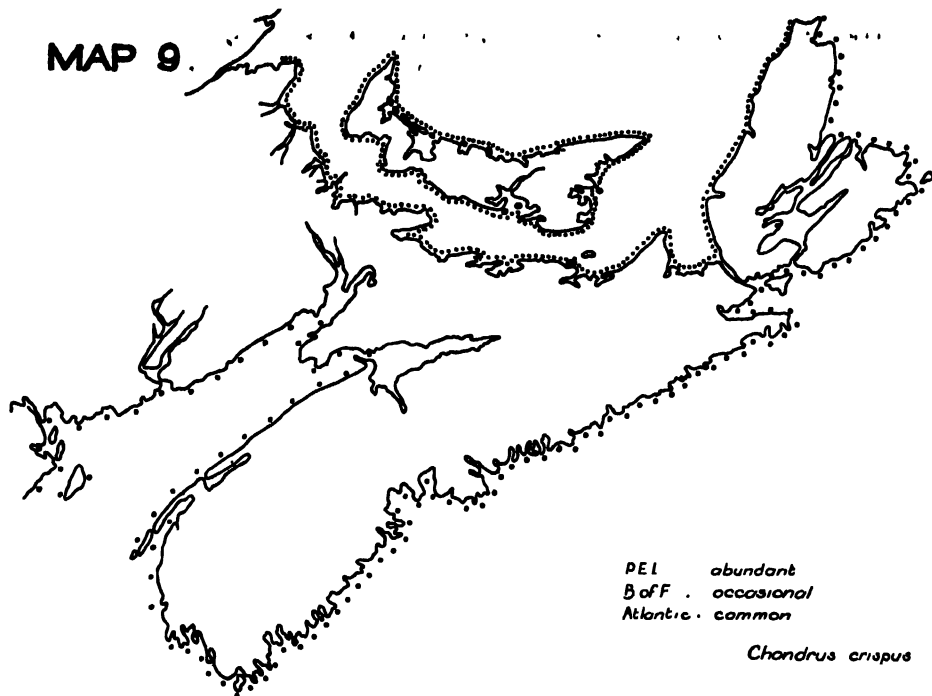
MAP 7



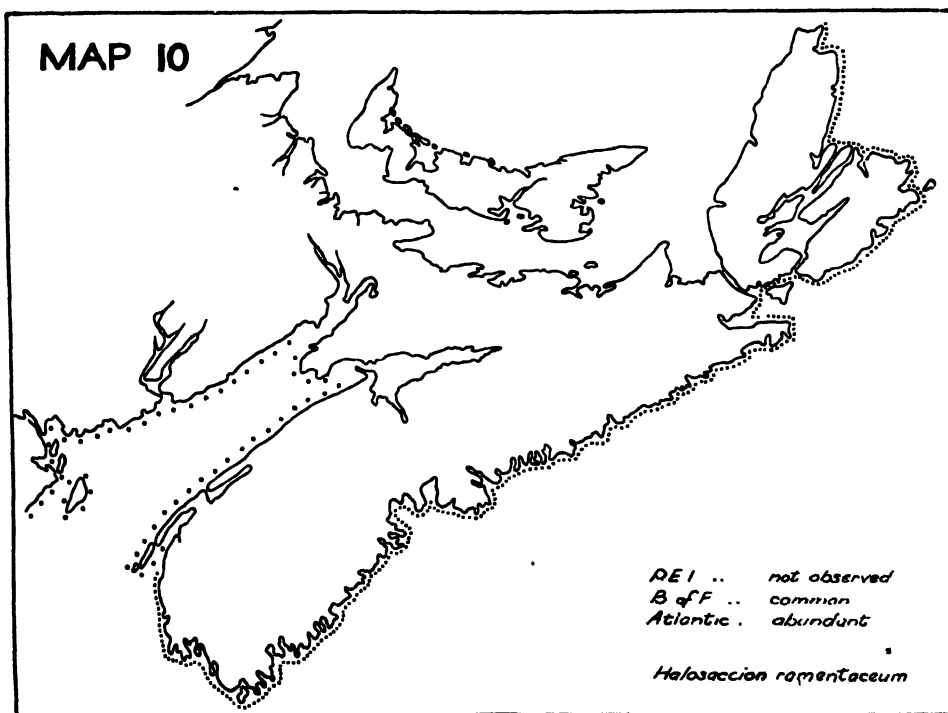
MAP 8



MAP 9.



MAP 10



# THE MARINE ALGAE OF THE MARITIME PROVINCES OF CANADA

## II. A Study of their Ecology<sup>1</sup>

BY HUGH P. BELL<sup>2</sup> AND CONSTANCE MACFARLANE<sup>3</sup>

### Abstract

The coastal area of the maritime provinces of Canada is divided into three regions, the Bay of Fundy, the Prince Edward Island, and the Atlantic. Each of these is distinctly different in regard to both marine flora and marine flora environment. The main features of the marine flora of each region are as follows: Bay of Fundy, generally dense and luxuriant; Prince Edward Island, a barren littoral zone and a rich sublittoral flora; Atlantic, intermediate in density and luxuriance with the predominance of large linear forms in the surf. With the exception of *Fucus vesiculosus* and *Ascophyllum nodosum* which are dominant in the littoral zones of both the Bay of Fundy and Atlantic regions, there are species dominant for each region and peculiar to it. The physical factors varying throughout the area, and associated with the floral differences are: water temperature, tides, wave action, clarity of the water as regards mud, structure and composition of the rocks along the shore, materials forming the ocean floor near the shore, slope of the intertidal zone, slope of the ocean floor near the shore, salinity, and ice action. Each of these physical factors is associated with certain characteristic features in the marine flora.

Marine algal associations have attracted the attention of algologists in many parts of the world, and have been discussed in many reports. Kjellman (10) in his report of the marine algae of the Arctic describes the various "formations." Cotton (2) gives a very clear picture of the formations and associations of the marine algae around Clare Island, Ireland. Others who have dealt with this subject in a similar manner are Hoyt (5) for Beaufort, N.C., U.S.A.; Johnson and York (9) for Cold Spring Harbor, N.Y., U.S.A.; Chater (1) for the Aberdeenshire Estuaries, Scotland; Johnson and Skutch (6, 7 and 8) for Mt. Desert Island, Maine, U.S.A.; Knight and Park (11) for the Isle of Man, England, and many more dealing with other parts of the world.

In comparison with these regions the ecology of the marine algae of the maritime provinces of Canada provides an even more interesting study, for this area is divided into three adjoining but distinctly different geographical and ecological regions. The regions are, (1) the coast bordering the Bay of Fundy, (2) the eastern Atlantic coast of Nova Scotia, and (3) the coast of Prince Edward Island together with the coasts of New Brunswick, Nova Scotia, and Cape Breton, bordering on the Northumberland Strait and the Gulf of St. Lawrence. As in Part I of this article these are called the "B. of F. Region," and the "Atlantic Region," and the "P.E.I. Region." In addition to the three main divisions of the area, there are the enclosed waters of the Bras d'Or Lakes. These salt water lakes exhibit conditions that are by no means typically marine, but the flora and physical conditions provide such striking contrasts, that they will be considered briefly. The unusual ecological

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situation that exists in the three main regions are well illustrated by Maps Nos. 3 to 10 inclusive, in Part I of this article. Throughout each region the flora is uniform in regard to dominant forms and character of growth, but each region is as distinct from the other as if separated by great distances and influenced by an entirely different climate and environment. A comparative study of the flora and its associated physical environment should reveal some important facts regarding the limiting factors in marine algal growth.

It is reasonable to assume that this distinct difference in the algal growth of each of the three regions is brought about by a difference in the physical environment in which the algae grow. As these regions are within a few miles of each other the general climatic conditions are similar, but other physical factors show wide variations. While collecting in each region an attempt was made to study the environment in which the algae were growing and to compare the environment of each region with that of the others. Certain important factors are uniform throughout the area, but many others vary extensively. Obviously it must be the latter which cause the difference in the algal growth of these three regions. The physical conditions that vary from one region to another are as follows; water temperature, tides, wave action, clarity of the water as regards mud, structure and composition of the rocks along the shore, materials forming the ocean floor near the shore, slope of the intertidal zone, slope of the ocean floor near the shore, salinity, and ice action. These factors are referred to in this same order all the way through the report, and are described in detail along with the flora for each region. The temperature quoted is that of the shore surface water of an exposed part of the coast, and represents the average temperature for the warmest months. Temperature and salinity are stated as approximations only and were calculated from the records in the possession of the Biological Board of Canada and from others given by M'Gonigle (12) in his report on the marine borers of the Atlantic coast of Canada.

Each region is described under the headings of general character of growth, dominant species, physical factors, and variations within the region.

### Bay of Fundy Region

#### *General Character of Growth*

The most striking feature of the Bay of Fundy region is the density of the vegetation and the luxuriance of the growth. The littoral zone is covered by a thick continuous mat of plants that start abruptly as a horizontal line of growth just below the upper limits of the littoral zone. The plants themselves are large. Average plants of *Ascophyllum nodosum* are from 30 to 40 in. long and some in St. John Harbor were found to be six feet, six inches. All the other forms are correspondingly large.

#### *Dominant Species*

The dominant forms in the upper parts of the littoral zone on all parts of the coast, and throughout the whole littoral zone in the more inland portions of the bays are *Fucus vesiculosus* and *Ascophyllum nodosum*. In the lower



littoral zone of all the more exposed parts of the coast the rocks are covered with a luxuriant growth of *Porphyra laciniata* and *Rhodymenia palmata*. The *Porphyra* plants are very large and dense in their form of growth, each one resembling closely a head of cabbage. Hanging from the crevices of the rocks and under the *Rhodymenia* plants are large bunches of a deep red *Polysiphonia urceolata*, and here and there extensive growths of *Cystoclonium purpurascens*. *Gigartina mamillosa* is abundant everywhere and in certain places forms a thick moss-like mat over the flat surfaces of the rocks. The most striking sublittoral species is the *Laminaria* which has so far been called *L. longicuris*, known locally as the "devil's apron." The plants of *Alaria esculenta*, *Desmarestia aculeata*, *D. viridis*, etc., are all exceptionally large. There are rich growths of *Euthora cristata* and *Ceramium elegans*, and dredging reveals extensive beds of such deep-water forms as *Delesseria sinuosa* and *Phylota pectinata*. In the tide pools large sheets of *Monostroma fuscum* are to be found everywhere, as are also large plants of the usual tide pool forms such as *Scytosiphon lomentarius*, *Dictyosiphon foeniculaceus*, *Ilea fascia*, *Enteromorpha linza*, etc. *Chondrus crispus* also grows in the tide pools, where it has a broad frond quite unlike the narrow deeply divided fronds of deeper waters. It is important to note some of the plants that are not common in this region. They are the typical sublittoral *Chondrus crispus*, *Laminaria agardhii*, *Ulva lactuca*, *Polysiphonia violacea*, and *Ceramium rubrum*.

#### Physical Factors

The physical factors are typical of a body of water where there is a big regular tidal movement of water into a funnel-shaped bay. The temperature of the water is very low, the average shore surface temperature of an exposed part of the coast for the warmest month being from 50 to 54° F. A big tidal rise and fall occurs twice every 24 hr. At North Head, Grand Manan Island, at the mouth of the bay this difference between high and low tides is from 21 to 24 ft., and at Amherst Harbor at the head of the bay, from 43 to 47½ ft. As a result of this great movement of water there are very rapid tidal currents and a constant mixing of the water. The ocean roll is lacking and except after a storm there is comparatively little surf. In the main part of the bay the water is clear, but at the head of the bay and at the heads of the inlets, the water is turbid. The rocks that line the shore and extend out under the water are a soft sandstone. These rocks crop out at various places under the water, but the ocean floor near the shore consists largely of mud. There are however, many places with sand, gravel, or larger loose rounded rocks. As a rule the slope of both the intertidal zone and the ocean floor near the shore is very gentle, giving an extensive intertidal zone and a broad zone of fairly shallow water below the low tide mark. The salinity ranges from about 3 to about 3.2. Owing to the fact that the tides do not let the water remain still long enough to freeze, very little ice is formed in the bay or its inlets.

#### Variations Within the Region

There are certain variations within the bay that are very striking. Proceeding from the mouth of the bay towards the head, the marine flora gradu-

ally becomes less in quantity and less luxuriant in growth, until it finally disappears in the vicinity of Blomidon on the Nova Scotian coast and a few miles north of Alma on the New Brunswick side. There are, however, scattered patches of algae inside these points especially where there are outcrops of rock on the ocean floor. This includes such places as the shores in the vicinity of Parrsboro where the flora is comparatively rich. Another outstanding exception is the whole tongue of land between Minas Basin and Chignecto Bay. At Spencer Island, Cape d'Or, Cape Chignecto and all the other places around Advocate the flora is very rich, though not as rich as near the mouth of the bay. The most striking fact is that this flora at Advocate has a slightly different aspect, the *Chondrus crispus* for instance is present in its typical marine form, which is quite different from the broad form found at the mouth of the bay. The water at the head of the bay occasionally becomes quite warm, especially when the tide has risen just after noon over sun-baked mudflats. Disregarding these few exceptions, the head of the bay consists of extensive mudflats destitute of any form of algal growth, either littoral or sublittoral and the water is muddy and at times it may be warm.

### Prince Edward Island Region

#### *General Character of Growth*

When it is considered that the Northumberland Strait is only about 15 miles due north from the head of the Bay of Fundy, the differences noticed in the marine flora of these two bodies of water are most striking. On the exposed parts of the coast throughout the whole P.E.I. region there is practically no algal growth in the littoral zone. In the bays and in the sheltered crevices all along the coast one finds stunted plants of *Fucus vesiculosus*, and here and there a few plants of *Ascophyllum nodosum*, but these plants are miserable and dwarfed specimens and to one accustomed to the rich B. of F. growth, they merely add to the barren appearance of the P.E.I. intertidal zone. One seldom finds either *Fucus vesiculosus* or *Ascophyllum nodosum* more than 8 or at the most 12 in. long. The only healthy forms forming a dense growth in this littoral zone are certain Greens that grow near the high water mark, such as small plants of *Enteromorpha intestinalis*. In contrast with this barren littoral zone there is usually a great quantity of loose weeds in the wash along the shore, and on examination it is found that there is a luxuriant sublittoral growth including many forms that are rarely found in other parts of the northwestern Atlantic.

#### *Dominant Species*

In view of these facts it is natural to expect that, in the P.E.I. waters, it is in the sublittoral zone that one finds the forms that give the region its distinctive character, and this actually is the case. On all the exposed parts of the coast there are extensive beds of the typically marine *Chondrus crispus*. The plants are large and comparatively free from epiphytic shellfish. This species grows in such quantities that it is washed up in great heaps\* all along the shore. An equally prominent form, but one that is found in localities

that are slightly less exposed, is *Fucus serratus*. The plants of this species occur in great abundance and are large and healthy. This is the only place from which it has been reported in the northwestern Atlantic. Other plants that are washed up in large quantities are, *Stypocaulon scoparium*, *Furcellaria fastigiata*, *Phyllophora brodiaei*, *P. membranifolia*, and *Laminaria phyllitis*. There are many others, but these are not only the most prominent, but occur in great abundance. It should be noted that none of these forms are common in either the B. of F. or the Atlantic regions. There are other forms that are not quite so abundant and yet are common for this region and either unknown or very rare in the other regions. These are *Chaetopteris plumosa*, *Gracilaria confervoides*, *Mesogloia vermicularis*, *Polysiphonia elongata*, *Stilophora rhizodes* and *Antithamnion cruciatum*. Each of these is abundant at certain places in the P.E.I. region. The *Antithamnion* at points such as Cavendish is found in great quantities as an epiphyte on *Chondrus* and *Corallina*. In addition to the distinguishing species found in this region, there are other ways in which the flora is quite distinct. Specimens of both *Ceramium* and *Polysiphonia*, for example, are apt to be quite light in color, and all the *Fuci* except *F. serratus* are very small. Other things could be mentioned, but these are sufficient to bring out the big difference between the marine flora of this region and that of any other part of the Canadian Atlantic coast.

The deficiencies in the marine flora in this locality are at once apparent to anyone accustomed to the ordinary conditions in the northwestern Atlantic. One at once notices the comparative rarity of *Ascophyllum nodosum* and *Alaria esculenta* that are so common everywhere else. *Laminaria longicruris* was not observed though dredging was carried out in likely places, and the *Laminaria* association includes an abundance of *L. phyllitis*, a form that is not found in either of the other regions. *Halosaccion* and *Dumontia* were not observed though they are common on other parts of the coast.

### Physical Factors

As might be expected there are exceptional physical conditions accompanying this unusual marine flora. The average surface temperature of the shore water at an exposed part of the coast for the warmest month is from 63 to 64° F., but the maximum is much higher than this. A number of records were made at points along exposed sections of the north shore of P.E.I., where the water is regarded as cold for that island, and it was not unusual for the water to reach 75° F., and at times it was as warm as 78° F. There are places on the south shore where at certain times the water becomes warmer than this.

The tidal movements of this region exhibit features that are strikingly atypical for a marine environment. They are entirely different from the tidal movements either on the Atlantic coast or in the Bay of Fundy. These features, although more marked on the north shore than on the south, are prevalent in the whole P.E.I. region. One characteristic of this peculiar condition is the irregularity of the change in level of the water from high to low tides, at times the difference in height being almost negligible. The maximum rise and fall is between seven and eight feet and the average is

about half this. But in every 24 hr. one of the tides provides very little change of level. This condition becomes extreme on the north shore of P.E.I. where during neap tides there are what are locally called "flat tides." That is, for one of the 12-hr. intervals during the day there is no apparent rise and fall. Sometimes collecting was continued at one place for more than four hours, during which it was not possible to detect any change in the level of the water. As a result of these conditions there are times when there is no regular alternating exposure and submersion of the littoral zone. During these periods the upper part of the zone may be exposed and the lower part submerged for over 12 hr. In marked contrast with this lack of rise and fall is the lateral movement of the water in a general direction parallel to the coast line. This movement takes place continuously, even during the extreme condition of a flat tide. At times the current is quite rapid and is especially noticeable at such places as North Cape, West Point, Borden, and East Point. Due to the regularity of the coast line, however, this lateral movement does not produce any rapid churning of the water such as is seen in the Bay of Fundy. The only exception to this is at East Point, which is the meeting place of two strong currents, flowing at right angles to each other. The tidal conditions described above are typical for the region and are predicted in the tide tables, but additional irregularities may be produced by a strong wind. These irregularities cannot be predicted and may result in making conditions even more unusual. At such times the lateral currents may become very strong and the level of the water kept for a number of hours either higher or lower than the normal. There is some mixing of the water by wave action, for especially on the north shore of P.E.I. there may be quite a surf during or immediately after a storm, but the ocean roll is lacking, hence there is not such a constant beating of the waves as on the Atlantic coast. Often indeed the water may be quite calm. As stated above, the tides do not produce the churning of the water found in the Bay of Fundy. Hence as is expected with these conditions of tide and waves, the water is clear.

The rocks all along the shore are shale or sandstone and are exceedingly soft and friable. There are of course muddy bays, but as a rule the shore is lined by fairly steep cliffs, and the intertidal zone is composed of mud, sand, or flat shelving rocks. These slope very gently, hence in spite of the small rise and fall there is still quite an extensive intertidal zone. The ocean floor near the shore is of a composition similar to the intertidal zone and also slopes very gradually. There is thus an extensive sublittoral area that supports the characteristic and luxuriant sublittoral growth. The extensive flat surfaces of the shelving rocks, with their numerous crevices, form ideal places for the growth of such forms as *Chondrus crispus*, *Corallina officinalis*, *Scytosiphon lomentarius*, *Chordaria flagelliformis*, etc. There is one feature of the rock formation that contributes towards the barrenness of the intertidal zone. The shape and structure of the rocks are such that there are practically no tide pools. This forms a marked contrast to certain parts of the Bay of Fundy and Atlantic coasts. The salinity of the water ranges

from about 2.6 to about 2.9. The salinity of the bottom water near shore is usually from 0.1 to 0.2 degrees higher than the surface water.

A winter condition which results from the various factors just mentioned is the drifting mass of ice formed throughout this region. There is not sufficient action of either tides or waves to prevent its formation. Hence it is formed every winter, and with the lateral movement of the water the ice moves slowly up and down the coast grinding against the exposed parts of the shore as it moves.

#### *Variations Within the Region*

There are a number of exceptions to the general rule within this region. In spite of the limited tidal rise and fall, and the uniformly straight coastline, there are a number of large bays that form tidal estuaries, where the water is so very shallow that, when the tide goes out, extensive mudflats covered with *Zostera marina* are left bare. There is usually a central channel through which the water continues to flow. Many of these places occur in this region. The ones visited most frequently during this survey were Bayview near Cavendish, P.E.I., Darnley Basin, P.E.I., and Caribou Harbor near Pictou, N.S. The marine flora of these places includes a number of forms not found elsewhere. These are *Gracilaria confervoides*, *Stilophora rhizodes*, and certain fine species of *Ceramium* and *Polysiphonia*. The most luxuriant growth for the region is found in the vicinity of Souris and East Point. The poorest growth is near the western end of the Strait of Canso in George Bay. There are all gradations between these two. As a rule, *Antithamnion*, the finer filamentous forms of *Ceramium*, and the species of *Laminaria* are more abundant in the slightly colder but more turbulent waters of the north shore of P.E.I. *Phyllophora membranifolia*, *P. brodiaei*, *Stypocaulon scoparium* and *Fucus serratus* are perhaps more abundant in the Northumberland Strait. The waters of the Gulf of St. Lawrence including the north shore of P.E.I. are slightly colder during the summer than the waters of the Northumberland Strait. Also on the north shore of P.E.I. the lateral current is more pronounced. At North Cape, P.E.I., there is a peculiar long narrow reef of round rocks. The rocks are anywhere from one to two feet in diameter. The reef is not more than 20 ft. wide and runs for about half a mile out into the Gulf. At a very low tide it is exposed for 300 or 400 yards and after that for a similar distance it is covered by not more than 3 or 4 ft. of water. This is quite different from anything seen anywhere else, and the flora also is different. It consists chiefly of brown annuals such as *Chorda filum*, *Chordaria flagelliformis*, *Laminaria agardhii* and *L. phyllitis*. A peculiar yellowish green form of *Rhodomenia palmata* is also found here. The most striking exception to the usual conditions in the P.E.I. region is the churning of the water at East Point, P.E.I. This is the one place in this region where the water is mixed violently. The tide flowing along the north shore meets the tide flowing along the eastern end of the island. As these enormous bodies of water are moving quickly at right angles to each other, the scene at East Point at certain times of the day is one of turmoil. At these times, stretching in a straight line

northeast from East Point and away out into the Gulf, is a narrow strip of churning rushing water.

### Atlantic Region

#### General Character of Growth

The part of this area most typically marine is the Atlantic region. It exhibits the fewest extremes in both flora and physical conditions. In most respects it is intermediate between the Bay of Fundy and the P.E.I. regions. There is considerable growth in the intertidal zone, but compared with that of the Bay of Fundy it is patchy. On certain types of shore and on rocks of particular formation the growth is abundant, while other long stretches are practically bare. In most places there is quite an amount of rockweed in the lower portion of the littoral zone, but the growth does not start abruptly as it does in so many places around the Bay of Fundy. Also plants of species such as *Fucus vesiculosus* and *Ascophyllum nodosum* are neither so large as in the Bay of Fundy nor so small as on P.E.I. The average and normal plants of *Ascophyllum nodosum* in the Atlantic region are from 18 to 30 in. long. The sublittoral growth is quite rich, and its appearance is quite different from that in either of the other regions, for at first it appears to consist chiefly of species of *Laminaria* of the long ribbon-like type.

#### Dominant Forms

The dominant forms in this region for the littoral zone are *Fucus vesiculosus*, *F. evanescens*, *F. platycarpus*, *Ascophyllum nodosum*, and *Porphyra laciniata*. Along with these but not quite so conspicuous though just as prevalent are *Enteromorpha linza*, *E. intestinalis*, *Monostroma fuscum*, *Hormiscia penicilliformis*, and *Bangia fusco-purpurea*. In the tide pools throughout the littoral zone, and on all the rocks at about the low water level and extending out for some distance under the water are extensive mats of *Ilea fasciata*, *Scytosiphon lomentarius*, *Chordaria flagelliformis*, and *Dictyosiphon foeniculaceus*. In the sublittoral zone the distinctive forms are *Alaria esculenta*, *Laminaria agardhii*, *L. digitata*, *Chorda filum*, *Desmarestia viridis*, *Halosaccion ramentacium*, *Poly-siphonia violacea*, *Chondrus crispus*, *Rhodomela subfusca*, *Ceramium rubrum*, etc. Of these sublittoral forms by far the most conspicuous species is *Laminaria agardhii*. At very low tide it can be seen festooned over all protruding rocks and at various places there are beds of this species where the plants are so large and dense that at low tide a 25-ft. sailing boat will be held as fast as though it had run aground. *Laminaria longicuris* that is so typical of the Bay of Fundy region is seldom seen on the Atlantic coast except when washed up on the shore. Actually it is present in fair quantities but grows out of sight in the deep and still water. *Rhodomenia palmata* is present in considerable quantities, but is very inconspicuous because it is usually tucked away in the crevices of the rocks. *Chondrus crispus* forms extensive beds below low water mark, but the plants are laden with mussels and other crustaceans. Thus with the scattered intertidal growth of *Fucus* and the heavy dark brown ribbon-like sublittoral forms, the algal growth of this region has a sombre appearance and is different in every way from either of the other two regions.

### *Physical Factors*

The physical factors of this region are fairly uniform from Cape Sable to Cape North. The average shore surface temperature for an exposed part of the coast for the warmest month is from 57 to 59° F. In the bays and harbors it becomes warmer, but there is no such variation as is found between the head and mouth of the Bay of Fundy. The tides are regular and the maximum difference between high and low tides is a little over six feet. The tidal currents are comparatively inconspicuous. The wave action is the one really extreme condition of this region. The ocean roll and the surf never cease and after a storm the force of the waves is terrific. The rocks along the shore are all hard, being granite, slate, or quartzite. The intertidal zone and the ocean floor near the shore are usually steep, rocky, and rugged. At certain places especially just northeast of Halifax there are shallow bays with mud-flats. At these places the ocean floor near the shore may slope gently for a certain distance. There are many long crescent-shaped sandy beaches. The salinity of the water ranges from about 2.9 to about 3.1%. Ice is not formed along the coast except in the enclosed bays, and unlike the drifting ice of the P.E.I. region, when once it is formed in these bays it remains stationary for the winter.

### *Variations Within the Region*

There are very few exceptions to be mentioned for this region. Mahone Bay is the largest body of semi-enclosed water in the region, and with its numerous islands and reefs, and rather extensive shallow water, it provides conditions not common for the rest of the coast, and a few forms of slightly more southern affinities have been found here. The marine flora of this bay should be studied more carefully. At certain other places the rock formation extends as a series of long reefs for a considerable distance out under the sea. It has been noticed that the seaweed in the wash near these shoal waters is unusually abundant. Whitehead, Tor Bay, Thrumcap, and Lockport are of this nature. Other observations have been made at Lockport that suggest that the flora there may be of special interest. A species of *Sargassum* was collected there and the *Chondrus crispus* found on the rocks in that vicinity is unusually free from attached shellfish. An intensive study of this district might prove profitable. There are without doubt other places in this region where variations from the normal conditions could be found. Where the bottom happens to be suitable for growth there is a very much richer flora than in places where conditions are unfavorable, but comparatively speaking the physical conditions and the marine flora are fairly uniform throughout the whole Atlantic region.

### **The Bras d'Or Lakes**

The Bras d'Or Lakes can be dealt with briefly. The marine flora is like that of a deep tide pool of the upper littoral zone. It includes such species as *Fucus vesiculosus*, *Ascophyllum nodosum*, *Scytosiphon lomentarius*, *Dictyosiphon foeniculaceus*, various species of *Enteromorpha* and *Cladophora* and

bleached forms of *Ceramium*. In the deep parts of the main lake forms like *Phyllophora membranifolia* may be found. The water of these lakes is fairly salty; thus they are regarded as part of the ocean, but unlike the ocean the physical conditions of these lakes might be described as "static." That is the temperature is consistently and uniformly warm during the summer, and the surface freezes over every winter. There is practically no tidal rise and fall, and no currents worth mentioning, except in the narrow channels connecting the lakes with the ocean. The surface is usually calm. Even the waves blown up during a storm are not large and soon die down. The shore may be mud, gravel, or rock. Naturally there is no intertidal zone, but a comparatively fixed waterline like an inland lake. The whole aspect of these Bras d'Or Lakes is the opposite of rugged. From a marine standpoint the outstanding characters are negative, and algologically, they are interesting because they lack a typical marine flora.

#### ASSOCIATION OF PHYSICAL FACTORS WITH FLORA

At the present it would be premature to attempt to arrive at definite conclusions regarding the part played by each physical condition in producing the distinctive flora in each of the regions. But it is profitable to record the cases where there is a consistent association or consistent lack of association between physical factors and a special type of flora.

##### *Temperature of the Water*

With the large and consistent difference in surface temperature existing in these three regions, it is inconceivable that some of the differences in the flora are not due to this factor. Setchell (14) has claimed that the maximum summer temperature can be used as a means to divide the algae of the ocean into regions, and he has drawn regional dividing lines for every five degrees difference in maximum summer temperature. However, this temperature factor is so conspicuous that one may be inclined to attribute to it greater importance than it deserves. One cannot help associating the warm waters of the P.E.I. region with the luxuriant growth of *Chondrus crispus*, and the presence of a distinctly southern species such as *Gracilaria confervoides*. On the other hand many of the species found in the P.E.I. region and not found in other parts of the maritime provinces are ones that are reported from the Arctic. *Furcellaria fastigiata*, *Stypocaulon scoparium*, *Chaetopteris plumosa* and *Fucus serratus* are common in the Arctic; yet they are found in the warm waters of the P.E.I. region but not in the cold waters of the Bay of Fundy. Again one is apt to assume that moderately warm water would be conducive to rich growth. If this were the chief factor involved the algae of the Bras d'Or Lakes would flourish. Instead they are sickly and stunted, and the region with the richest growth is the Bay of Fundy where the water is the coldest. Competing with the luxuriance of the cold Bay of Fundy is the sublittoral growth of the P.E.I. region where the water is warmest for the area. Hence it is obvious that too much importance should not be placed on temperature as a limiting factor in the distribution of marine algae.



### Tides

The tides are responsible for two sets of conditions to which the algae give a definite physiological response. These are the regular alternating exposure and submersion, and the churning of the water due to tidal currents. Exposure appears to be associated with the healthy growth of certain forms of which *Rhodomenia palmata* is an example. Between tides in the Bay of Fundy the part of the shore where it grows may become quite dry. On the Atlantic the intertidal zone is so narrow that due to the spray from the waves the lower part where the *Rhodomenia* grows is seldom quite dry. The plants of this species found on the Atlantic coast are not in such a healthy condition as those that grow in the Bay of Fundy. At Cape North, P.E.I., and at the mouth of Halifax Harbor in the Atlantic region, *Rhodomenia palmata* was found growing where it was constantly submerged. In both cases the plants were poorly developed, a light yellowish green in color, and badly infested with epiphytes. This and similar evidence with other species would suggest that considerable and regular periods of exposure may be a necessary condition for the healthy growth of certain forms. The absence of healthy intertidal growth in the P.E.I. region may possibly be associated with the irregularities of the tides. However, this is one of the points regarding which it is too early to make a definite statement.

On the other hand there appears to be no doubt regarding the influence of the tides when they cause a mixing and churning of the water. Where clear water is kept stirred and churned right from the surface to the bottom, there the growth is always luxuriant. The whole outer part of the Bay of Fundy region is an example of this. In the P.E.I. region the area around Souris and East Point is the same, while the undisturbed waters of the Bras d'Or Lakes provide a negative example in this respect, suggesting that the scarcity of algal growth in these lakes is due to a certain extent to the complete lack of tidal currents. So many instances such as these were observed and the evidence was so convincing that it is important to stress the constant association between a mixing of the water and a luxuriant growth of marine algae.

### Wave Action

Severe wave action is associated with the luxuriant growth of forms of a certain type and the absence of forms of another type. Nearly all the species growing luxuriantly in the surf are linear in shape. There are the ribbon-like and cord-like forms such as *Laminaria agardhii*, *Chorda filum*, *Chordaria flagelliformis*, etc. These apparently thrive in the surging waves and are torn off only during a storm. In among the stipes of these forms and even on the exposed rocky surfaces that are beaten by the waves are delicate filamentous forms such as *Polysiphonia violaceae*, *Antithamnion floccosum*, etc. Just below the low water mark there is usually a band of a very wiry *Halosaccion ramentaceum*. All these species are linear or filamentous in structure. Broad forms like *Laminaria longicuris* and *Rhodomenia palmata* are absent from the exposed rocky surfaces in such a locality. It is probable that they cannot stand the force of the waves, whereas the species with a linear form

provide very little resistance to the surging water. This condition is typical of the whole Atlantic coast where the continuous heavy surf is the only extreme condition of the environment. In contrasting the physical factors and the associated flora just described, with the situation found in the Bay of Fundy, one is justified in associating the lack of continuous severe wave action in the Bay with the abundance of such broad flat forms as *Laminaria longicuris* and *Rhodymenia palmata*.

#### *Nature of Rocks Along the Shore*

As far as could be observed the kind of rocks did not have very much effect on growth. Other conditions being favorable, the growth was rich regardless of the type of rock. It was thought that perhaps the algae would have difficulty in becoming established on a smooth surface of a hard rock, so a boulder of smooth hard rock was rolled from above the tide level to a position in fairly turbulent water. Within a month this stone was covered with growth. There is one condition, namely, the friable character of the rocks around P.E.I., which may be a limiting factor in the growth of certain plants. These rocks are so soft that fairly strong holdfasts such as those produced by a *Fucus* will pull away lumps of rock before the stipe or holdfast of the plant will break. This may account for the fact that in the P.E.I. region there are no large plants on any part of the shore subjected directly to the action of the waves. Of course where the shore is soft mud, such as at the head of the Bay of Fundy, there is no algal growth.

#### *The Slope of the Intertidal Zone and the Slope of the Ocean Floor Near the Shore*

The relation between the slope of the zone of growth and the richness of the flora appears to be obvious. Most seaweeds do not flourish on a perpendicular surface, and practically all the species grow better if the surface is somewhat sloping. The most important consideration in this respect is the area of the zone where it is possible for algae to grow. Although marine algae may grow at considerable depths, the largest part of the sublittoral growth in the area considered is found within about 12 ft. of the surface of the water at low tide. The zone of growth is thus from high water mark to about 12 ft. below low water mark. Where the surface is perpendicular the zone is very narrow and the area of growth is small. Where the surface is sloping the area is greatly increased and in addition it provides a more favorable place of attachment for the majority of forms. So naturally the most abundant growth occurs where the intertidal zone and the ocean floor near the shore slope gently. This gently sloping condition is characteristic of the whole of the Bay of Fundy and the P.E.I. regions. In the Atlantic region there are a number of places, such as Lockport, where this condition prevails, and as already mentioned it is at these places one finds the most abundant growth.

#### *Salinity*

So far as the three main regions are concerned, there is no reasonable suggestion of any association between the salinity of the water and the distinctive character of the flora of each region. There is no doubt that a very low

salinity inhibits marine algal growth. There is a splendid illustration of this at St. George, New Brunswick. One reaches St. George by an inlet that is about four miles long and from a quarter of a mile to a hundred yards wide. The salinity of the water grades from normal at the mouth to pure fresh water near the falls at the head. The plants of *Fucus vesiculosus* are large and normal at the mouth of the bay. They continue to grow right up to the fresh water, but become smaller and smaller, till those at the upper limits are but little protuberances on the rocks. There are without doubt other conditions involved, but the salinity appears to be the chief factor. Evidence like this could be multiplied and would suggest that salinity has a decided local influence on algal growth. However it is difficult to attribute the diverse conditions of growth in the three regions of this area to differences in salinity. The difference of 0.2% between the average salinities of the Bay of Fundy and Atlantic regions seems insufficient to cause any great difference in growth, for there is a greater variation than this within each region. The P.E.I. region has a lower salinity, but the forms that flourish there are species that in other parts of the world are typical of places where the salinity is normal. The slight difference in the salinity of the surface water and the bottom water near the shore seems hardly enough to cause the big difference between the scanty littoral and the rich sublittoral growth. A more intensive and comparative study of local conditions in limited and contrasting areas is necessary before one could draw any conclusions.

#### *Ice Action*

Due to the conditions already described in the paragraphs for each region, ice appears to have very little influence on the growth of the algae in either the Bay of Fundy or the Atlantic. But the exposed rocks in the P.E.I. region provide evidence indicating that there the ice is a very important factor. On a point of land, the rock surface facing the open bay is exposed to the action of the laterally drifting ice. Any crevice in the rock provides a surface not subjected to the moving ice, yet in these crevices the wave action is more severe. Studying the rocks all along the shore from this standpoint, the surfaces that are scraped by the moving ice are bare, but the surfaces that are protected from the ice action, even though they might be subjected to many other destructive agencies, are more or less covered with some form of growth. This evidence suggests that the lateral movement of the large mass of floating ice may be one of the factors responsible for the barren intertidal zone in the P.E.I. region.

It is important to restate the object of this summary. Its purpose is to bring out the great differences in the marine flora of the three main regions in this area, and to place on record the ways in which the differences in marine flora appear to be associated with physical conditions. These observations were made during a survey of the marine algae of the Atlantic maritime provinces of Canada. It is not intended to make any broad deductions from this evidence, for it is felt that a more intensive study of certain regions, and some experimental work are necessary before this could be done, but these three regions provide such striking contrasts that the area as a whole is an ideal situation for ecological investigation.

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# GELASINOSPORA, A NEW GENUS OF PYRENOMYCETES WITH PITTED SPORES<sup>1</sup>

BY ELEANOR SILVER DOWDING

## Abstract

*Gelasinospora* is a genus closely related to *Sordaria*, characterized by the foveolate sculpturing of the spore wall. At present it contains two species, as follows:—

I. *Gelasinospora tetrasperma*, a coprophilous fungus which has been cultivated from spores collected in Manitoba and in Ontario.

The species is described, together with illustrations of the chief characters, such as the four-spored condition of the ascus and the thickened ring bordering its apical perforation.

Each normal-sized spore gives rise to a homothallic mycelium. The normal spore contains four nuclei. Asci very occasionally produce dwarf spores. Each dwarf spore gives rise to a mycelium which produces archicarps, but no perithecia. The mycelia from dwarf spores fall into two groups, (+) and (−), in regard to their sexual behavior. When (+) and (−) mycelia are paired, perithecia are produced. Sometimes asci contain giant spores. The giant spores usually produce homothallic mycelia and the spores usually contain six nuclei.

II. *Gelasinospora cerealis*, isolated from the crown of wheat and oats in Manitoba.

The species is described, together with illustrations of the chief characters, such as the eight-spored condition of the ascus and the two radial thickenings at its apical perforation.

Each spore gives rise to a homothallic mycelium. The spores are binucleate.

## I. Diagnosis of Genus and Species

Two previously undescribed species of Pyrenomycetes have recently come to the attention of the writer, both species being characterized by the presence of strikingly pitted sculpturing on the spore walls. On account of the spore markings, the genus has been named *Gelasinospora*, from "gelasinos" (γελαινος), a dimple. *Gelasinospora* is closely related to two other genera, *Neurospora* (the red bread-mold fungi), and *Sordaria*.

*Gelasinospora* gen. nov.: Perithecia sparsa vel conferta, conica vel pyriformia, fusca vel atra; rostro conoideo, brevi; asci stipitati, aparaphysati, apice perforato; sporidia hyalina, demum fusca, denique atro-opaca; episporio eleganter et regulariter foveolato; macroconidia et microconidia absentia. Species typica, *G. tetrasperma*.

The spores of *G. tetrasperma* and *G. cerealis* are flattened slightly (Text-figs. 3a and 9). Foveolate markings are most easily observed in the young spores. When examining the mature spores, it is necessary to use a strong light on account of their black color. It is even more satisfactory to crush the spores between a slide and cover slip, when the pits show very distinctly on the fragments of the walls (Text-fig. 4, a and b; Plate III, Fig. 8).

*Gelasinospora tetrasperma* spec. nov.: Perithecia sparsa, erumpentia, atra, opaca, pyriformia, membranacea, 0.3 × 0.6 mm., basi hyphis radiantibus aincta; rostro glabro, conoideo; asci plerumque tetraspori, interdum 1-, 2-, vel 3- spori, cum giganteis sporis, raro 5-spори cum nanis sporis, 8 × 230μ, aparaphysati, permanentes, cylindranei, apice asci truncato, perforato, ore circumvento crasso annulo; ascospori plerumque 13.2-16.0μ × 20-28μ,

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interdum  $15.0-19.8\mu \times 38.0-42.9\mu$  vel  $10.0-13.2\mu \times 16.5-18.0\mu$ , visi a dorso ellipsoidei vel ovoidei, visi a latere plani vel fabiformi, hyalini primo, viridi-atri serius, foveolati; species homothallica.

On ptarmigan and rabbit dung. Manitoba and Ontario.

Distinctive characters: four-spored asci with thickened rim about apical perforation.

*Gelasinospora cerealis* spec. nov.: Perithecia sparsa, erumpentia, fusca, translucida, subglobosa, membranacea,  $0.3-0.4$  mm.  $\times$   $0.6-0.7$  mm., basi sparsis hyphis cincta, rostro glabro, cylindrico; asci octospori,  $29.7-33.0\mu \times 214.5-260.0\mu$ , paraphysati, permanentes, cylindracei; apice asci truncato, perforato, incrassato radiatim in duobus locis; ascospori  $23.1-25.0\mu \times 26.4-32.0\mu$ , visi a dorso late ellipsoidei vel sub globosi, visi a latere plani vel fabiformi, interdum apiculati, hyalini primo, atri serius, foveolati; species homothallica.

Isolated from the crown of wheat and oats. Manitoba.

Distinctive characters: eight-spored asci with two radial thickenings near the perforation; subglobose spores, occasionally apiculate at one end.

Cultures of *G. tetrasperma* and *G. cerealis* have been deposited with the Centraal Bureau voor Schimmelcultures, at Baarn.

## II. Material and Methods

### (1) *Gelasinospora tetrasperma*

In 1932 this fungus was first obtained from ptarmigan droppings collected by Mr. Wm. Güssow, in Manitoba about 30 miles north of Fort Churchill on Hudson Bay. The following winter, in Ottawa, the dried dung was moistened, and in less than a week four or five perithecia developed which were the source of the cultures used. Spores from the perithecia were transferred to culture media and the fungus has been kept in cultivation at Ottawa for the last three months.

The writer described the new species to Dr. R. F. Cain of the University of Toronto, who is engaged in making a special study of the Sordariaceae of Ontario (1). He stated that Mr. J. Savage in 1931 collected rabbit dung near Thunder Bay on the north shore of Lake Superior in Ontario, and when this material was moistened in the laboratory a similar Pyrenomycete with pitted spores developed upon it. The fungus from Ontario is not in culture at present, but permanent slides of the perithecia and spores show that it is apparently identical with the fungus collected at Hudson Bay. We may therefore assume that *G. tetrasperma* occurs in Ontario as well as in Manitoba.\*

\* As this paper goes to press, the writer has received from England cultures of "*Sordaria fimicola*, (four-spored form)" from Miss Page. Miss Page (14), has shown that her fungus exhibits the same sexual and nuclear condition that the writer has found in *G. tetrasperma*. Miss Page's species is foveolate-spored, and is identical in appearance in every other way with *G. tetrasperma*. In both fungi there are normal-sized bisexual spores, and, occasionally, dwarf spores which produce heterothallic mycelia. Four monosporous mycelia of *G. tetrasperma*, two of (+) and two of (-) reaction, all derived from dwarf spores, were each paired with a (+) and with a (-) mycelium of dwarf-spore origin from the four-spored *S. fimicola*. No perithecia were formed on any of the eight paired mycelia. The writer considers Miss Page's *S. fimicola* to be *G. tetrasperma* even though it is sexually incompatible with the Canadian *G. tetrasperma*. The English cultures were cultivated from soil which was collected by Mrs. H. S. Williamson in 1930 at Oxshott.

## (2) *Gelasinospora cerealis*

This species has been collected so far only from Manitoba. It was isolated by Dr. J. E. Machacek in 1932, from the diseased crown of oats growing at Souris, Manitoba, and also found in the diseased crown of Durum wheat at Deloraine. Dr. G. R. Bisby, of the University of Manitoba, had examined with much interest the cultures of *G. tetrasperma* submitted him by the writer. He sent in return Dr. Machacek's culture, which resembled *G. tetrasperma* so closely that he considered that it might possibly be included in the same genus. The fungus was subsequently named *G. cerealis*.

*G. cerealis* was obtained by Dr. Machacek from the diseased cereal plants by stripping away the culms and roots from the underground part above the seed, and after washing and surface-sterilizing these parts, they were plated out on agar that was still warm.

*G. cerealis* may be one of the organisms causing foot rot, or it may be a soil-inhabiting fungus occurring occasionally as an accidental saprophyte on cereal crowns.

## (3) *Spore Deposits*

To obtain a spore-deposit of *G. tetrasperma* or *G. cerealis*, a glass plate is suspended an inch or two above the ripe perithecium for about an hour, so that spores discharged from the perithecium form a deposit against the under surface of the glass. When the spores of either species are discharged from the ascus they do not cling together as in *Pleurage anserina* (5), but become separated from one another in the air, so that in a spore deposit it is impossible to recognize sister spores (Text-fig. 6).

In a spore deposit of *G. cerealis* there is some variation in spore size, but in *G. tetrasperma* the variation is extreme. Most of the spores of *G. tetrasperma* are of constant size (about  $25\mu$  long), but there is occasionally, once among about 200 spores, a giant spore about  $40\mu$  long, and still more rarely, once among about 2,000 spores, a dwarf spore only about  $17\mu$  long (Text-fig. 6).

## (4) *Culture Methods*

In order to obtain monosporous mycelia, spores were removed one by one from a spore deposit by the aid of a needle (8) and transferred singly to sterilized tubes of culture medium. The mycelia were found to grow readily on malt agar, prune agar, potato-dextrose agar, cornmeal agar and horse-dung agar. Throughout the investigation malt agar was used for the culture medium.

Dodge (2) has shown that spores of many coprophilous Ascomycetes germinate more readily after they are heated. The writer had sent cultures of *G. tetrasperma* to Dr. Dodge, and relative to these cultures he writes from New York "not a single ascospore germinated after twelve hours or so." (He had sown spores of *G. tetrasperma* on cornmeal agar.) "I then submitted the

spores to a temperature of about sixty degrees for three-quarters of an hour, and much to my surprise obtained a high percentage of germination."

Spores shaken up in sterile water were poured over two malt agar plates. One plate was left unheated and the other was heated in an oven for half an hour, until a temperature of 52° C. was reached, and slowly allowed to cool. It was found that about three times as many spores germinated in the heated as in the unheated plate.

The experiments about to be recorded on the sexuality of the spores of *G. tetrasperma* and *G. cerealis* were carried out before the expedient of heating the spores had been tried. The unheated spores gave sufficiently high germination for the purpose, for out of 100 spores sown separately on agar slants without being heated, 26 germinated.

When a Petri dish is inoculated with the mycelium of *G. tetrasperma* or *G. cerealis*, hyphae grow out rapidly from the inoculum at a rate of about 2 mm. per hour, so that after 24 hr. the mycelium has grown over the surface of the agar to the periphery of the dish. It then forms aerial mycelium at the sides of the dish. In *G. tetrasperma* the aerial mycelium is very abundant and is flesh-pink in color. In *G. cerealis* it is not so abundant and it is white except after being collected together with needle or forceps, when it appears pale pink. In about three days minute archicarps appear over the surface of the agar and on the aerial mycelium on the glass, and in about five days the archicarps have developed into mature perithecia. The two species of *Gelasinospora* thus complete their life history in less than a week.

### III. The Genus *Gelasinospora*

#### (1) *Comparison with Sordaria*

*Gelasinospora* resembles *Sordaria* very closely. The perithecia of *S. fimicola* Ces. and De Not., which may be considered the type species of *Sordaria* (Plate I, Figs. 1 and 3), and the perithecia of the two species of *Gelasinospora* (Plate II, Fig. 1; Plate III, Fig. 1) are all superficial, smooth, short-beaked, dark colored, membranaceous, and pyriform. The asci of both genera are pored (Plate II, Fig. 7; Text-fig. 9). The eight-spored *G. cerealis*, and the eight-spored species of *Sordaria*, as far as they have been investigated, are homothallic (9). The two genera differ in that the spores of *Sordaria* are smooth and possess a mucilaginous sheath, while those of *Gelasinospora* are pitted, and possess no sheath (Plate I, Fig. 3).

Spores of the two species of *Gelasinospora* have been examined at all ages within the ascus, and also after discharge. Discharged spores have been examined in various media, water, glycerol, lactic acid, iodine, and India ink, and in air. Of the spores examined in air, some were from old spore deposits and some were recently discharged and still wet. For illumination an open diaphragm, a partly closed diaphragm, and a dark-field condenser were each tried in turn. The spores of *S. fimicola*, which are provided with a distinct sheath (7, 13) (Plate I, Fig. 3), were used for comparison, and from these



examinations nothing could be seen on the spores of either of the species of *Gelasinospora* that could be described with certainty as a mucilaginous sheath.

After the spores of *S. fimicola* are stained in gentian violet, the mucilaginous sheath takes on a deep violet color. The spores of *G. tetrasperma* and *G. cerealis* when similarly treated show no such violet sheath. When spores are examined in air or in liquid, with the light cut down to a narrow beam, a shining band about  $1\mu$  wide appears about the spore. This band was finally interpreted as being due to refraction.

The spores of *G. tetrasperma* and *G. cerealis* possess some protoplasmic or mucilaginous material which clings to them after their discharge. It appears as an occasional thin fringe of granular material, stained by gentian violet. This material appears to cement the spores to any object to which they adhere, because if spores are removed from a spore deposit by a needle, they leave a circular imprint of some refractive substance on the glass (Plate II, Fig. 10). It is probable that any species of spore which is discharged through the pore of an ascus is similarly lubricated; but such a scanty, indistinguishable covering could not be taken for a mucilaginous sheath in the sense that this term is used by systematists to define the genus *Sordaria*.

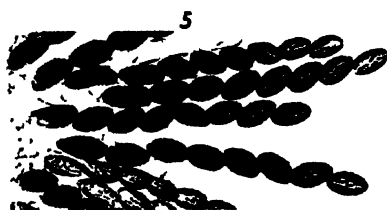
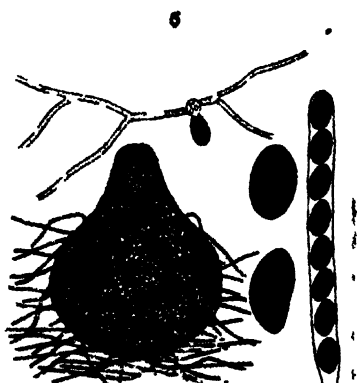
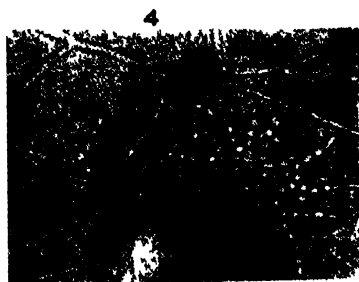
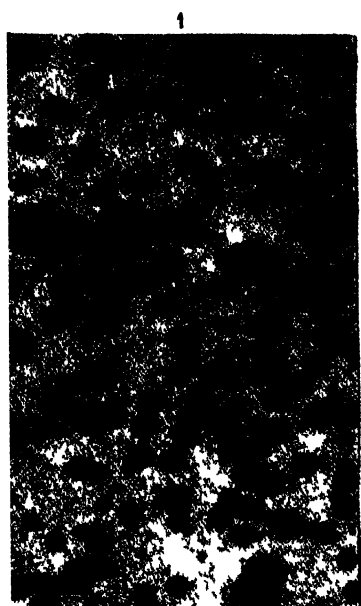
## (2) Comparison with *Neurospora*

Members of the genus *Neurospora* Shear and Dodge possess superficial, black perithecia similar to those of *Gelasinospora*. They possess asci with a single pore. Hyphae occupy the central cavity of the young perithecium, at the time the first asci are being developed, but in the mature perithecium there are no paraphyses. *Neurospora* can be distinguished from *Gelasinospora* by the longitudinal nerve-like markings on the spore wall (Plate I, Fig. 5), and by the possession of macroconidia (Plate I, Fig. 4) and microconidia. It differs from *Gelasinospora* also in that the eight-spored species (*N. crassa* Shear and Dodge, and *N. sitophila* Shear and Dodge) are heterothallic (3, 17), while the eight-spored *G. cerealis* is homothallic.

The two four-spored species, *G. tetrasperma* and *N. tetrasperma* Shear and Dodge, are similar in that both normally produce bisexual spores, but occasionally produce dwarf spores which produce heterothallic mycelia. Dr. B. O. Dodge in 1932 (4) had obtained a fertile interspecific hybrid in the genus *Neurospora* and he suggested that the writer attempt to produce an intergeneric hybrid by mating the dwarf spores of *G. tetrasperma* and *N. tetrasperma*. Acting upon this suggestion, seven monosporous mycelia of *N. tetrasperma* and five monosporous mycelia of *G. tetrasperma* were obtained from dwarf

## EXPLANATION OF PLATE I

FIG. 1. *Sordaria fimicola*—a malt agar culture producing perithecia. Photograph by Gussow.  $\times 10$ . FIG. 2. *S. fimicola*—pored ascus containing eight spores. After Griffiths.  $\times 440$ . FIG. 3. *S. fimicola*—perithecium, and spore with mucilaginous sheath. After Griffiths. Perithecium,  $\times 65$ ; spore,  $\times 575$ . FIG. 4. *Neurospora sitophila* Shear and Dodge—macroconidia. After Shear and Dodge.  $\times 185$ . FIG. 5. *Neurospora sitophila*—asci, each containing eight spores with nerve-like markings. After Dodge.  $\times 300$ . FIG. 6. *Anthostomella destruens* (*Melanospora destruens*) showing perithecium, ascus, and spores. After Shear. FIG. 7. *Ceratostoma avocetta* (C. and E.) Sacc.—showing perithecium, ascus with paraphyses, and spores. The perithecium of this species is often buried in the wood. After Clements and Shear. Perithecium,  $\times 80$ .





spores. The 12 mycelia were paired in all possible combinations. No perithecia were produced when mycelia of the two species were grown together, even after two months, although perithecia were produced when mycelia were mated which were of the same species but of different sexual groups. The attempt to obtain a hybrid between *N. tetrasperma* and *G. tetrasperma* was therefore unsuccessful. Furthermore, the paired mycelia of different genera exhibited no reaction whatever which could be interpreted as a sexual response.

### (3) *Comparison with Anthostomella and Ceratostoma*

*Anthostomella* is distinguished from *Gelasinospora* by the persistently submerged habit of the perithecia, and the possession of paraphyses.

*Anthostomella destruens* Shear (15, 16), was first isolated in 1907 from the pulp of a diseased cranberry grown in New Jersey. Shear himself does not consider *A. destruens* to be typical of *Anthostomella* because it has no paraphyses, and to judge from his figures (Plate 1, Fig. 6), the perithecia are superficial, not submerged. It has recently been included in *Melanospora*, but it is doubtful whether the new classification is more satisfactory. *M. chionea* (Fr.) Cda., which has been considered the type species of *Melanospora*, has a grey perithecium with a long slender beak, fimbriate at the apex, in marked distinction from the dark-colored, short-beaked perithecium of *A. destruens*.

The writer examined preserved specimens of perithecia and spores of *A. destruens*, kindly sent by Dr. Shear. To add to his description (16), the perithecia are superficial, and clothed in a web of hyphae. They are black and opaque except in a strong light when they appear brown and translucent except for the beak. The spores in one view appear flattened.

Mason (10) examined American and English cultures of *A. destruens* for microconidia. He states, "In spite of repeated efforts, phialides have been seen neither in the isolation maintained at the Lister Institute nor in the present culture," (isolated from a rotten apple at London).

In all the characters mentioned by Shear (16) and in those above described, *A. destruens* agrees with *Gelasinospora*.

*A. destruens* differs from *Gelasinospora* only in the possession of smooth-walled spores. After examining large numbers of the smooth spores of *A. destruens*, the writer discovered one spore which was distinctly pitted.

From these observations it is concluded that of the Pyrenomycetes allied to *Gelasinospora*, *A. destruens* is the most closely related species known.

The genus *Ceratostoma* is similar to *Gelasinospora* in the superficial habit of the perithecium, but is distinguished by its long beak (Plate I, Fig. 7). Dr. Shear drew the writer's attention to a fungus described by Nichols (12) as *C. brevirostre*, but which was later considered not to be Fries species. From Miss Nichols' description of the rapid growth of the fungus, the abundance of perithecia, and the absence of conidia, one might place her species very near to, if not within, the genus *Gelasinospora*.

(4) *A Previously Described Pyrenomycete with Pitted Spores*

In 1897, Mouton (11) described and figured the spores of a *Pyrenomycete* collected only once from Beaufays near Liège, Belgium, on charred sawdust. To the knowledge of the writer, it is the only recorded *Pyrenomycete* with pitted spores and they are said to be "elegantly, and regularly foveolate." Because of its coriaceous perithecium the fungus was placed in the genus *Rosellinia*, and it was named *R. calospora* on account of its beautiful spores. If this species is rediscovered it must then be decided whether or not it should become a member of the genus *Gelasinospora*.

IV. *Gelasinospora tetrasperma*(1) *Description of Species*

The perithecia of *G. tetrasperma* are black, and even under the strongest light, completely opaque. They are shown in Plate II, Figs. 1, 3, 4 and 11. The hyphae which clothe their base are brownish (Plate II, Fig. 1).

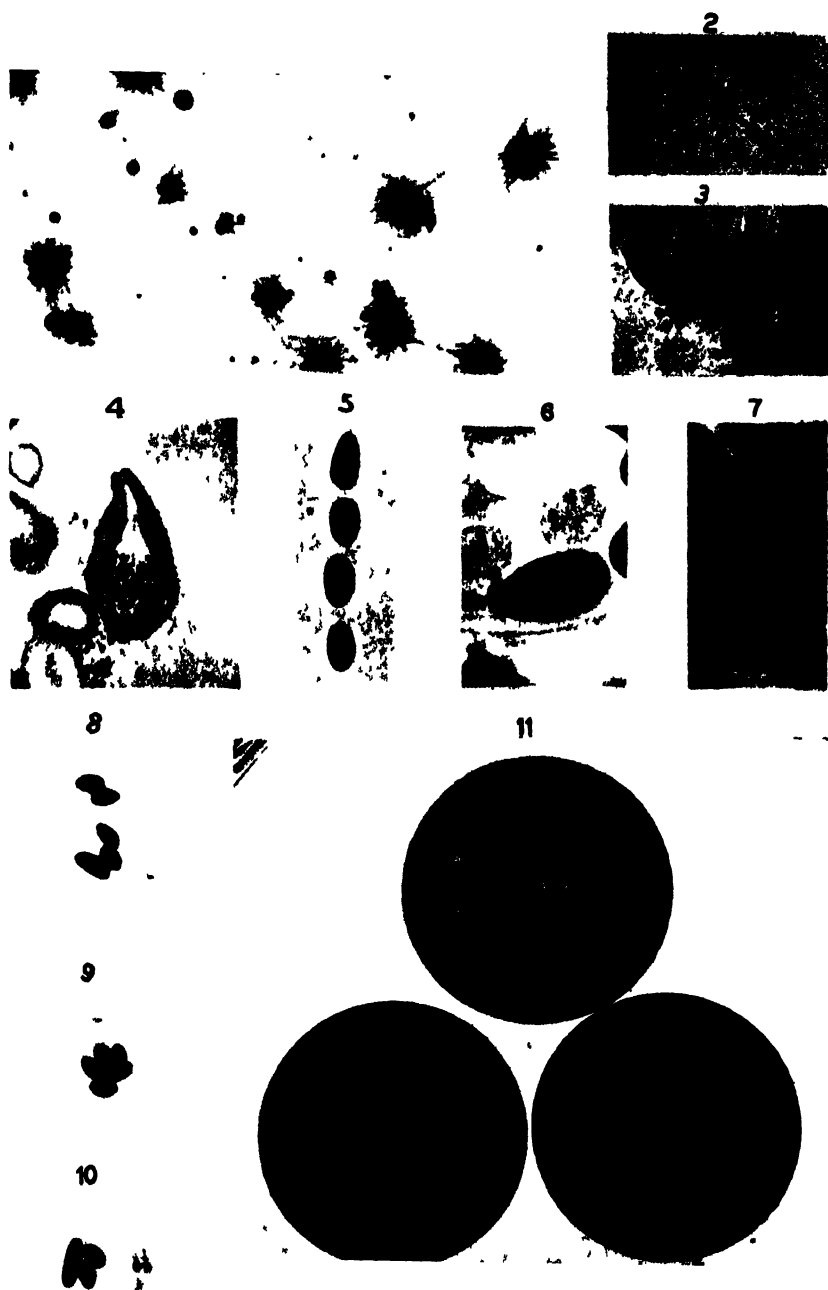
Photographs of normal asci are shown in Plate II, Figs. 2 and 5. The stipes, which are not illustrated, are moderately long and slender. Reference to Text-fig. 7 will show the different types of asci that have been observed. There are figured: A, a four-spored ascus with normal spores; B, asci with fewer than four spores, containing normal and giant spores; C, asci with five spores, containing normal and dwarf spores; D, asci with four spores, containing normal, giant and dwarf spores. The ascus pores bordered with mucilaginous bands are sketched in Text-fig. 5, *a*, *b*, and *c*, and a section of a perithecium in which the bands about the perforations have been stained in safranin is photographed in Plate II, Fig. 7. The gelatinous band also stains brilliantly in cotton-blue dissolved in lactic acid. Faull (6) describes and illustrates a similar gelatinous rim about the ascus perforation of *Neurospora crassa*.

The spores, although black in ordinary illumination, are distinctly green in a strong light. Their shape is shown in Text-figs. 3, 5 and 6 and in Plate

## EXPLANATION OF PLATE II

*All figures are those of Gelasinospora tetrasperma*

FIG. 1. A malt agar culture, producing perithecia. The perithecia are surrounded at their base with aerial hyphae.  $\times 15$ . FIG. 2. A group of asci in water, obtained by crushing a perithecium between a slide and coverglass. At the upper left is an ascus, containing two normal spores and one giant spore. There are no paraphyses.  $\times 40$ . FIG. 3. Crushed perithecia.  $\times 15$ . FIG. 4. Section of a perithecium, stained, showing spores and paraphyses.  $\times 40$ . FIG. 5. Four young spores in an ascus. Pits are clearly shown on the spore walls.  $\times 300$ . FIG. 6. A ripe spore within the perithecium, showing pitting.  $\times 625$ . FIG. 7. Section of a perithecium, stained, showing the tips of five asci. The three uppermost apices show pores bordered by thickened bands which have stained deeply.  $\times 625$ . FIG. 8. A deposit of five normal-sized spores.  $\times 180$ . FIG. 9. A deposit of four normal, and one dwarf spore.  $\times 180$ . FIG. 10. A deposit of two normal, and one giant spore. To the right is a gelatinous impression left by a group of spores which have been rubbed off the glass.  $\times 180$ . FIG. 11. Above, a plate of malt agar which has been inoculated with a single normal spore. The mycelium is a week old and has produced perithecia. Left, a similar culture of a mycelium from a single dwarf spore. The mycelium is a month old, and is sterile. Right, a plate inoculated with two mycelia derived from two dwarf spores. Perithecia were formed where the two mycelia met, and now, when the culture is two weeks old, they have spread over one-half of the plate.  $\times$



*Gelasinospora tetrasperma spec. nov.*



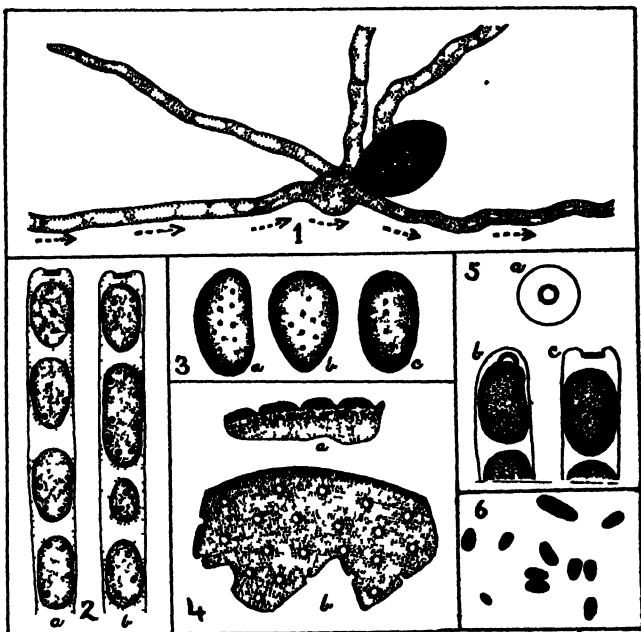
II, Figs. 5, 8, 9 and 10. Although some are elliptic, others are strongly ovate. The spore pits are photographed in Plate II, Figs. 5 and 6, and sketched in Text-fig. 4, *a* and *b*. The spores germinate from one end only, where a primary vesicle is first produced (Text-fig. 1). The mycelium produces no macroconidia.

As phialospores are frequently found in fungi related to *Gelasinospora* (10), a careful search was made for microconidia in *G. tetrasperma*. The fungus was grown on the following media: malt agar, potato-dextrose agar, prune agar, corn-meal agar, horse-dung agar, and on both filter-paper and soil impregnated with horse-dung decoction. The mycelium was examined microscopically on the agar plate on which it was growing, and mounted in water between a slide and cover slip. Sterile water was poured over the aerial mycelium of cultures and the water was then examined under the microscope.

No microconidia were ever discovered from these examinations.

## (2) The Sexuality of the Normal and Abnormal Spores

The results obtained from experiments on the sexuality of the spores of *G. tetrasperma* agree with those reported from similar work on other four-spored Ascomycetes with abnormal spores. In 1927, Shear and Dodge (17) and Dodge (3) showed that the normal and giant spores of *N. tetrasperma* produce mycelia which are all capable of giving rise to perithecia, while the dwarf spores produce mycelia which remain sterile unless appropriately mated. In 1931, the writer (5) obtained the same results with *Pleuroge anserina*. In 1933, Page (14) working with "*Sordaria fimicola* (four-spored form)" found that the normal and abnormal-sized spores of this species behaved similarly.



TEXT-FIGS 1-6 *Gelasinospora tetrasperma* Camera lucida drawings of spores. Magnification FIG 4, 1,350, FIG 6, 60, all other figures, 300 FIG 1. Germinating spore. The spore has produced a vesicle at one end which has given rise to five hyphae. Protoplasm and vacuoles are moving in the direction of the arrows. FIG 2. Young asci stained in safranin and light-green, to show the nuclei in the spores: a, an ascus containing normal spores, each with four nuclei; b, an ascus containing abnormal spores. The giant spore possesses six nuclei, the dwarf spore, two. FIG 3. Mature spores: a, spore in lateral view; b, ovoid spore in face view; c, ellipsoid spore in face view. FIG 4. Fragments of the wall of a crushed spore, showing pits: a, pits in profile; b, pits in face view. FIG 5. Apices of asci, showing ascus perforations with thickened margins: a and b, face views; c, side view. FIG 6. Spores from a spore-deposit. At the top is a giant spore, at the lower left a dwarf.



To determine the sexuality of the normal-sized spores of *G. tetrasperma*, 26 normal spores were sown separately on slants of malt agar and induced to germinate. Every one of the 26 monosporous mycelia produced perithecia in less than a week. Plate II, Fig. 11, top, shows a monosporous culture derived from a normal spore. The culture was a week old and was producing perithecia. From 26 such cultures it is concluded that the normal-sized spores are bisexual.

After sowing a number of dwarf spores separately on agar slants, five monosporous mycelia of dwarf-spore origin were obtained. The five mycelia were periodically transferred to fresh medium and kept for three months. At the end of that time every one of them remained sterile. After the first week there appeared, upon all the cultures, archicarps barely visible to the naked eye. These bodies eventually became greyish in color but never developed into perithecia. Plate II, Fig. 11, left, shows a monosporous culture a month old, derived from a dwarf spore. The mycelium has produced no perithecia.

The five mycelia, Nos. 1, 2, 3, 4, and 5, were then mated to one another in all possible ways by planting pairs of inocula side by side on malt agar slants. From the 15 matings it was found that mycelia Nos. 1 and 2 remained sterile when mated with each other, that mycelia Nos. 3, 4, and 5 were sterile when mated with one another, but that mycelia Nos. 1 or 2 when mated with mycelia Nos. 3, 4 or 5 produced perithecia in less than a week.

Plate II, Fig. 11, right, shows a Petri dish in which a pairing has been made between the two mycelia, 1 and 3. When the two mycelia met, perithecia first appeared along the line of contact. The perithecia-producing band subsequently widened and spread over one half of the dish. It would appear from the uneven distribution of the perithecia on the agar in the photograph, that one mycelium has invaded the territory of the other and there initiated the production of perithecia. From 15 similar paired cultures, it is concluded that the five mycelia derived from dwarf spores fall into two groups on the basis of their sexual reactions. Mycelia Nos. 1 and 2 belong to one group, and Nos. 3, 4 and 5 to the other group. The mycelia are all morphologically alike but are heterothallic in the sense that it is necessary to have the co-operation of two mycelia belonging to different physiological groups to produce perithecia.

Ten monosporous mycelia derived from giant spores were then grown on agar slants. In less than a week nine of the ten mycelia had produced perithecia. We may, therefore, assume that giant spores of *G. tetrasperma* are usually bisexual.

The one sterile mycelium derived from a giant spore was kept three months and it still remained sterile. The mycelium was then mated with each of the five mycelia derived from dwarf spores. It produced perithecia when it was mated with "dwarf mycelia" of one sex, but remained sterile with mycelia of the opposite sex. The one exceptional giant spore must, therefore, have given rise to a mycelium similar to that from a dwarf spore.

### (3) *The Number of Nuclei in the Normal and Abnormal Spores*

In order to obtain material with which to make permanent sections of perithecia, agar plates were inoculated with mycelia of *G. tetrasperma*. Eight or nine days after inoculation blocks of agar with perithecia upon their surface were cut out of the plate, immersed for one minute in Carnoy's fluid, and transferred to Flemming's fluid (medium strength), where they were allowed to remain for 24 hr. The material was then washed in tepid water for two and one-half hours, dehydrated, imbedded in paraffin, cut by microtome, and stained with safranin and light-green.

After this treatment, in the very young spores the nuclei could be distinguished by their red color. In the older spores the pigmented wall made it impossible to observe the protoplasmic contents.

#### B

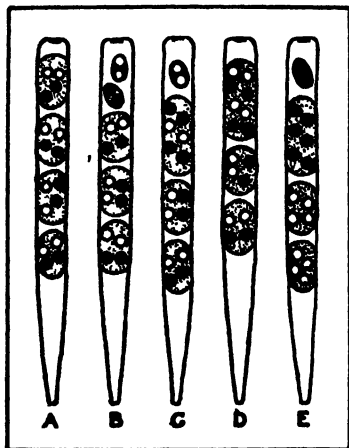
TEXT-FIG. 7. *Gelasinospora tetrasperma*. Diagram of the different types of asci actually observed: A, normal ascus containing four spores, B, asci containing less than four spores; C, asci containing five spores, D, asci containing four spores, some of which are abnormal in size.

It was found that *normal* spores of *G. tetrasperma* contain *four* nuclei (Text-fig. 2, a). After considerable search, five or six sufficiently young dwarf spores were discovered. The *dwarf* spores each contained *two* nuclei. About 12 young *giant* spores were found. Each contained *six* nuclei. It is possible that there are also giant spores which contain 8 or even 16 nuclei. Such giant spores are shown in Text-fig. 7, B.

It happened that in one section there was an abnormal four-spored ascus which contained one dwarf, one giant and two normal spores. The dwarf spore contained two nuclei, the giant six, one normal spore four, while in the other normal spore two nuclei could be seen distinctly and two less distinctly (Text-fig. 2, b). It is likely that young abnormal asci, as well as young normal asci, always contain 16 nuclei, because we have no reason to suppose that an abnormal delimitation of spores is ever accompanied by an abnormal number of cell divisions.

The results of the experiments upon the sexuality of the normal- and abnormal-sized spores, and the corresponding variation in the number of nuclei within the spore, go to show that the nuclei within a mature ascus may be of two kinds, which we might designate as (+) and (-). Spores which produce fertile mycelia may contain *both* (+) and (-) nuclei; spores which

produce sterile mycelia may contain *either* (+) or (-) nuclei. Text-fig. 8 shows five of the many possible arrangements of (+) and (-) nuclei in different asci.



TEXT-FIG. 8. *Gelasinospora tetrasperma*. Diagram representing five of the possible ways in which nuclei might be arranged in the ascus. Nuclei of one "sex" are shown white, of the other black: A, normal ascus with four spores all capable of producing fertile mycelia; B, ascus containing two dwarf spores, which would produce mycelia that would remain sterile unless mated together; C, ascus with a dwarf spore, which would produce a sterile mycelium, and a giant spore with four (+) and two (-) nuclei; D, ascus with a giant spore with four (+) and four (-) nuclei; E, ascus with a dwarf and a giant spore, the mycelia of which would both be sterile.

## V. *Gelasinospora cerealis*

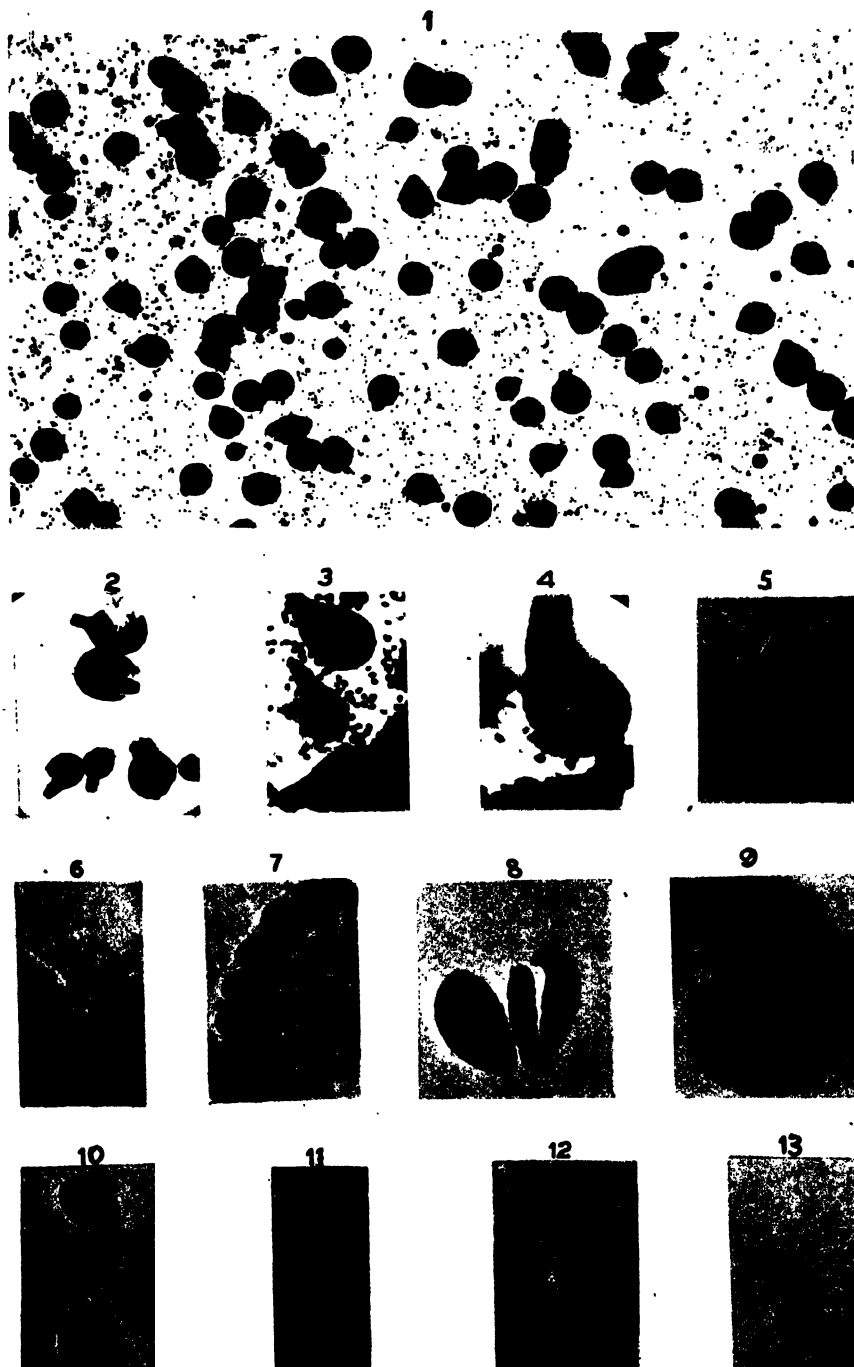
### (1) *Description of Species*

The perithecia of *G. cerealis* usually appear black (Plate III, Figs. 1 and 2), but when examined with a strong light they are brown and faintly translucent (Plate III, Figs. 3 and 4). They are almost spherical in contrast with the more pyriform perithecia of *G. tetrasperma*. The perithecia contain periphyses as shown in Plate III, Fig. 5, but no paraphyses. Plate III, Figs. 6 and 7, and Text-fig. 9 show the asci with their eight spores. When the crushed perithecia are stained in cotton-blue in lactic acid, there can be seen the apical perforations of the asci with the two radial thickenings (Text-fig. 9). The spores are much wider in proportion to their length than those of *G. tetrasperma*. They are black, or, in strong light, brown. Some of the spores are remarkably apiculate at one end (Text-fig. 9). The shape of the spores is also shown in Plate III, Figs. 6, 7, 9, 12, and 13. The pitting of the epispore in *G. cerealis* is sometimes closer than

### EXPLANATION OF PLATE III

All figures are those of *Gelasinospora cerealis*

FIG. 1. A malt agar culture, producing perithecia. Spores which have been shot away are lying on the surface of the agar.  $\times 10$ . FIG. 2. Perithecia crushed in water.  $\times 15$ . FIG. 3. Translucent perithecia revealing asci within.  $\times 20$ . FIG. 4. Perithecium showing structure of wall.  $\times 30$ . FIG. 5. Section of young perithecium, stained, showing spores and periphyses.  $\times 30$ . FIG. 6. A group of asci in water, obtained by crushing a perithecium between a slide and cover-glass. There are no paraphyses.  $\times 30$ . FIG. 7. Eight young spores in an ascus. Pits on the spore walls are clearly shown.  $\times 225$ . FIG. 8. Ripe spore, crushed in water to show pits.  $\times 600$ . FIG. 9. Young spores showing pits. The upper spore is orientated to show one of the germ-pores.  $\times 600$ . FIG. 10. Two young spores, the lower of which is abnormal in that it possesses ridges on the spore-wall instead of pits.  $\times 225$ . FIG. 11. Spores on agar, one of which (centremost of upper three) is germinating.  $\times 30$ . FIG. 12. Germinating spore which has sent out hyphae from both ends.  $\times 225$ . FIG. 13. Germinating spore which has sent out a hypha from one end.  $\times 225$ .



*Gelasinospora cerealis* spec. nov.



... *G. tetrasperma* (Cf. Plate II, Fig. 6, and Plate III, Fig. 8). When the spores germinate, some of them produce vesicles from one end only, but most of them produce vesicles from both ends (Plate III, Figs. 12 and 13). The mycelium produces neither macroconidia nor microconidia.



TEXT-FIG. 9. *G. cerealis*. Drawing (semi-diagrammatic) of ascus containing eight spores. Near the perforation, at the apex of the ascus there are two radial thickenings. Three of the spores are strongly apiculate. The lowermost spore is turned so that it appears bean-shaped.  $\times 250$ .

## (2) The Sexuality of the Spores

To determine the sexuality of the spores of *G. cerealis*, 18 spores were sown separately on slants of malt agar and induced to germinate. Five days later every one of the 18 mycelia had produced perithecia. Plate III, Fig. 1, represents a monosporous culture which has been growing in a Petri dish for about 10 days, and is producing perithecia. From 18 such cultures it is concluded that the spores produce homothallic mycelia.

## (3) The Number of Nuclei in the Spores

Using the same method applied to *G. tetrasperma*, sections of perithecia of *G. cerealis* were obtained and stained in safranin and light-green. The spores of *G. cerealis* proved to be less favorable material for cytological examination than those of the preceding species, but very young spores were eventually found which revealed the fact that the spores of *G. cerealis* each contain two nuclei.

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## SEED CONTENT, SEEDLING PRODUCTION AND FRUITFULNESS IN APPLES<sup>1</sup>

BY W. H. BRITTAIN<sup>2</sup> AND C. C. EIDT<sup>3</sup>

### Abstract

The behavior of different types of apple crosses was found to be very similar, whether or not emasculation was practiced, indicating that selfing was not a serious factor in the latter case. In both cases it was found that (1) all varieties were fruitful when diploid varieties were used as male parents, though triploid  $\times$  triploid crosses sometimes approximated the latter in this respect, and that (2) all varieties, regardless of chromosome constitution, were less fruitful when triploids were used as male parents. Though triploid varieties had a lower seed content than diploids, the seed content within the variety was characteristically greater wherever diploids were used as pollen parents. The results obtained with respect to seedlings were in general agreement with those based on seed alone, the order of production being as follows: first, diploid  $\times$  diploid; second, triploid  $\times$  diploid; third, diploid  $\times$  triploid; and fourth, triploid  $\times$  triploid. No correlation between seed content and weight could be demonstrated in Gravenstein, Baldwin, King and Wagener and a barely significant correlation in the case of Northern Spy. Various morphological abnormalities resulting from imperfect pollination are described.

### Introduction

A number of workers have pointed out the relation that exists between development of seed and the measure of success of the cross. Some data from experiments on the production of viable seedlings are also available, though most of these experiments have been made from the breeding standpoint. Bach (1) has noted that the number of seeds in an apple is definitely influenced by the pollen parent. Musser and Andrews (19) report that the number of seeds per apple is a good index of the value of the pollen parent; Burrell and Parker (3) found the relative value of various pollen varieties on McIntosh to be indicated by seed counts. That the number of viable seeds is generally lower in fruits resulting from selfing has been stated by Johansson (13). Crandall (4) showed that self-pollinations were generally unsatisfactory both in the number of fruit produced and in the number and vigor of seedlings

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The data in this paper were obtained in part in the course of an investigation of the pollination of the apple, carried out under the chairmanship of Mr. Arthur Gibson, Dominion Entomologist, and in part in the course of an apple breeding investigation conducted as part of the regular work of the Dominion Experimental Station, Kentville, Nova Scotia.

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resulting from such self-pollinations. Howlett (11) working with Stayman Winesap found no significant difference between the seed content of fruit produced by various pollinizers. The same worker was also unable to find any significant difference in seed germination between effective and ineffective pollinizers. Latimer (15) found a notable difference in the ability of different pollens to cause seeds to develop in McIntosh. Crane and Lawrence (5) state that often a single seed is sufficient for development in apples and that even it may be imperfect and therefore fruitfulness may be obtained in spite of a high degree of generational sterility.

According to many other workers imperfectly fertilized apples tend to be one-sided and several have presented evidence to show that there is a correlation between weight and number of seeds (Müller-Thurgau (18); Ewert (7); Heinicke (10); Kobel (14); Latimer (15, 16); Einset (6). Einset found a coefficient of correlation amounting to  $0.237 \pm 0.059$  between weight of fruit and number of filled seeds in Gravenstein and of  $0.349 \pm 0.055$  when all seeds, empty and filled, were considered. His calculations were based on only 118 fruits.

It may be observed that of all commercial varieties studied Gravenstein would be least likely to show such a correlation, since this variety gave the smallest average number of seeds and the largest percentage of seedless apples. Further evidence on this point is presented later. Ewert (8, 9), in a consideration of the subject of parthenocarpy in apples, contends that the parthenocarpic development of fruit can be encouraged by an abundance of available food. By preventing all the blossoms from being pollinated he was able to produce seedless fruit as large as normal specimens.

Kobel (14) has noted the tendency of varieties with a low pollen germination and irregular chromosome distribution to produce few or no seeds. He describes as "false parthenocarpy" the condition that obtains when fertilization has actually taken place, but, owing to the irregular chromosome distribution, further seed development does not occur.

It has been pointed out by Einset (*loc. cit.*) and also by other workers (Heinicke (10), MacDaniels and Heinicke (20)) that normal seed production is so intimately connected with the physiological processes of the fruit, that apples with developed seeds have an advantage in the competition for water and organic nutrients over those with fewer or no seeds, in that the presence of seeds is necessary for the normal development of the conducting tissue in the fruit that connects it with the tree.

The foregoing review, though incomplete, will serve to give an indication of the present status of opinion with respect to the problem that forms the subject of this paper.

### Normal Seed Content of Standard Varieties

The type number of embryos in the apple fruit is ten, though with Northern Spy, Deacon Jones, and possibly other varieties, double this number appears

to be typical. Few individual apples, however, produce the maximum number of seeds and the average is far below this figure. The following table shows the average number of seeds produced by four standard varieties over a period of three years, the figures being based on a count of 500 drop apples and 200 picked apples of each variety for each of the three years. Drop apples refer to those that come off in the so-called "June drop," though "July drop" would be more descriptive of the situation in Nova Scotia.

TABLE I

AVERAGE NUMBER OF SEEDS\* IN DIFFERENT VARIETIES BASED ON COUNTS FROM 16 ORCHARDS

Variety	1928		1929		1930		Average	
	Drop	Picked	Drop	Picked	Drop	Picked	Drop	Picked
Gravenstein	2.22	3.75	1.98	3.19	2.54	3.60	2.24	3.35
King	4.15	5.60	3.55	5.40	3.93	5.99	3.85	5.55
Baldwin	3.80	5.45	3.53	5.54	4.45	5.78	3.96	5.62
Spy	5.61	8.04	5.53	8.58	5.90	8.36	5.67	8.37

\*Seed refers to total seed whether filled or not.

It will be seen that the results are reasonably uniform, Gravenstein producing fruit with the smallest number of seeds, Spy the largest. In all varieties the average number of seeds in the drop fruit is less than in the picked, though individual drop fruit sometimes have more seeds than certain individual picked fruit. It is worthy of note that the first three varieties are triploid forms having 51 instead of the normal 34 chromosomes, and are typically few-seeded.

In the case of tented Gravensteins unsupplied with bees it was possible to produce seedless fruits as large as or even larger than normal ones, but the number so produced was less than where a satisfactory pollinizer and bees were supplied and in no case was a commercial crop of apples secured. Perfectly formed Gravensteins, as large as or larger than the average, containing no seeds whatever are commonly found in commercial orchards. Gravenstein is the only variety tested by the writers that showed this tendency, though others have been recorded. However, many of these seedless Gravensteins are abnormal in other respects as will be explained elsewhere. It is interesting to contrast the behavior of another triploid, *viz.*, Baldwin, which rarely, if ever, produces seedless fruit and which, when selfed, produces an average of over three seeds per fruit. The fact that Baldwin is self-compatible, whereas Gravenstein is quite self-incompatible may, in conjunction with triploidy, explain the foregoing fact. On the other hand, such varieties as Spy, tented under the same conditions, sometimes produced no fruit and, in all cases, a very small percentage set was secured, while seedless apples were not found in these tests.

The following tables, showing the seed content of certain standard varieties with both diploids and triploids used as male parents, have been compiled

from results of our pollination studies. These tables are based on four years results and are presented to show the massed results from these two types of crosses. More detailed varietal analyses are presented later.

TABLE II

AVERAGE NUMBER OF SEEDS OF STANDARD VARIETIES WHEN DIPLOIDS ARE USED AS MALE PARENTS

Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit	Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit
Gravenstein	16959	1739	3.34	Baldwin	14585	1433	5.31
King	16132	527	5.35	Spy	14971	1698	9.38
Golden Russet	5862	305	6.03	Blenheim	6181	420	4.65
Cox Orange	13423	1647	6.43	Stark	4376	454	3.88

TABLE III

AVERAGE NUMBER OF SEEDS OF STANDARD VARIETIES WHEN TRIPLOIDS ARE USED AS MALE PARENTS

Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit	Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit
Gravenstein	8768	61	2.31	Baldwin	9046	783	3.70
King	8643	213	3.60	Spy	10129	262	4.60
Golden Russet	11896	249	3.74	Blenheim	3195	124	3.96
Cox Orange	13125	429	4.29	Stark	3669	186	2.92

### Sources of Evidence

The evidence on which the present study is based, comes from two sources, as follows:—

1. The results from breeding work in which a large number of crosses were made, the blossoms being first emasculated.

2. Results obtained from pollination studies, the object of which was simply to test the value of the pollen of different varieties as a means of improving the set of the variety used as a female parent. In these studies emasculation was neglected in order to perform as large a number of pollinations as possible. The limb unit method was used in this work and ten replications of each cross, each on a different tree, were made. The limbs selected were as uniform as could be secured and the trees themselves of uniform age, size and treatment.

All the pollinations were made with pollen previously prepared, and applied to the stigmas as soon as the receptive stage was reached, and, as far as possible, before their own anthers had dehisced. Seed counts from the resulting apples are now available over a four-year period and counts of seedlings resulting from various crosses for two years. In order to make a proper

comparison the two-year figures only are used in the detailed tables. A summary table, showing the four-year results is also given, omitting seedling records.

It is recognized that, from the standpoint of the present paper, possible selfing constitutes a source of error, but it is considered that this does not prevent the results from being significant, since the technique employed tended to reduce selfing to a minimum. Selfing tests conducted under optimum conditions show most of the varieties employed to give remarkably low sets. An exception is Baldwin, and for this reason the figures from all crosses in which Baldwin occurs as a female parent are eliminated from our calculations or shown separately. Furthermore, the results from these tests, with respect to the percentage fruit, seed and seedlings obtained from the various crosses, are consistent with the results obtained from emasculation tests and indicate that the conclusions arrived at on the basis of a study of the data presented in our pollination experiments alone, would be essentially the same as though based on data from experiments in which emasculation was practiced. This seems to show that the final results obtained in the pollination work were not seriously interfered with by selfing. They are therefore presented as being strongly corroborative of those secured in the course of breeding work, in which emasculation was employed throughout.

The data available from the pollination studies are derived from two different sources:—

(a) Results from hand pollination tests with six standard varieties for five years as regards fruit and seed production. In this series fruit and seed data for four years were available, but seedling production for two years only.

(b) Results of various hand pollinations made with Blenheim and Gravenstein as female parents. In these experiments fruit and seed production are also considered, but in this case the seed has been classified, whereas in the other tests the total seed count is used, excluding only the undeveloped seed.

(c) In addition to the foregoing a tabulation has been made to show directly the degree of fruitfulness in relation to seed content.

(d) In order to show the relation between seed content and weight, a correlation between these two factors has been calculated for several standard varieties.

(e) In order to show relation of seed content to premature drop and certain abnormalities of the fruit, data from a series of Gravenstein trees enclosed beneath tents under different conditions of pollination, together with certain other data, are included.

#### 1. *Evidence from Crosses Performed in Breeding Experiments (Series 1)*

These results were obtained in connection with breeding experiments with apple varieties. All trees were enclosed beneath tents and emasculation and hand pollination performed in the usual manner. All blossom clusters were reduced to two blossoms per spur and obviously weak spurs were eliminated. This accounts for the fact that, between Series 1 and Series 2, in the effective

crosses, the former shows a higher percentage success than the latter, in which only the centre bloom was removed and all laterals crossed.

The crosses are classified on the basis of their chromosome constitution, *vis.*, (1) diploid  $\times$  diploid, (2) diploid  $\times$  triploid, (3) triploid  $\times$  diploid and (4) triploid  $\times$  triploid. In addition, as a matter of interest, results from other crosses involving varieties of unknown constitution, but believed to be either diploids or triploids on the basis of their behavior have been included. It may be stated at this point that the possession of pollen of high germinability and good quality for crossing purposes, with relatively high seed and seedling production, appears to be associated with diploidy and the opposite condition with triploidy.

## 2. *Evidence from Crosses Obtained in Pollination Tests (Series 2), on Standard Varieties*

These tests were made on non-emasculated blooms, as already described, in the course of pollination experiments with standard varieties of apples. The method of tabulation and terms used are exactly the same as in tables in Series 1. In showing results of triploid  $\times$  triploid crosses in the detailed tables, figures are given both with and without Baldwin as a female parent. This is because of the high percentage of self-fruitfulness of this variety, which it was considered might affect our results. The figure excluding Baldwin as a female parent is therefore used when discussing general results for crosses of this type. Figures on all points except seedlings are available for the five-year period, 1928-1932 inclusive. Figures including seedlings are available for the years 1930-1931 inclusive. These two sets of figures are given separately to allow for better comparison with the series averages, which only cover the years 1930-1931.

## 3. *Evidence from Pollination Tests on Gravenstein and Blenheim (Series 3)*

Some evidence may be gleaned from the writers' records of certain crosses made on Blenheim and Gravenstein in 1929. The seed from these two crosses was secured and classified, the percentage fruit and seed obtained from the original blossoms pollinated was calculated, as well as the average number of seeds per fruit for all crosses. Since both these varieties are triploids the results of these crosses are of some interest, since they enable us to study the results in a large number of crosses of both the triploid  $\times$  triploid and triploid  $\times$  diploid type, with respect to fruitfulness and the relation of seed production to fruit setting. Unfortunately the chromosome number of all the varieties used as male parents is not known. From their behavior it is believed that most of the varieties of unknown constitution will prove to be diploids.

## 4. *Evidence Obtained from a Study of the Seed Content of Various Crosses*

In order to bring out more clearly the influence of the pollen parent upon the seed content of the resulting fruit and its relation to fruitfulness, all the fruit obtained from certain standard varieties was classified according to its seed content and the results tabulated to show the degree of fruitfulness obtained with the different seed counts. These data are presented in Table V.

TABLE IV  
STANDING OF DIFFERENT TYPES OF CROSSES IN SERIES 1, 2 AND 3

Type of cross	Per cent fruit				Per cent seeds per fruit				Per cent seeds				Per cent seedlings			
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3a</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3a</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3a</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3a</sub>	S <sub>3</sub>
Diploid × diploid	23.24	12.11	11.50	—	7.59	7.76	7.09	—	176.4	75.70	77.41	—	115.85	—	38.52	—
Diploid × triploid	0.68	2.89	1.56	—	6.67	4.23	4.04	—	4.50	9.93	6.02	—	2.70	—	5.17	—
Triploid × diploid	28.46	10.46	9.45	7.41	3.28	4.08	4.41	4.44	93.37	36.79	—	—	20.86	34.06	8.29	—
Triploid × triploid (excluding Baldwin)	3.15	2.72	2.10	0.41	2.00	3.31	3.39	1.22	6.29	6.81	5.62	—	1.05	—	0.83	—
Triploid × triploid (with Baldwin)	—	—	4.71	—	—	—	3.47	—	—	—	13.49	—	—	—	2.91	—
Triploid × unknown*	—	—	—	7.23	—	—	—	4.91	—	—	—	—	—	—	—	—
Diploid selfed	—	1.66	—	—	—	4.36	—	—	—	6.83	—	—	—	2.98	—	—
Triploid selfed	—	3.56	—	—	—	3.11	—	—	—	9.04	—	—	—	2.07	—	—

S<sub>1</sub>, average for 1927-1932; S<sub>2</sub>, average for 1930-1931 only; \*Unknown varieties, probably all diploids; — No data.

TABLE V

TABLE SHOWING PER CENT FRUITFULNESS OF CROSSES IN RELATION TO THE AVERAGE NUMBER OF SEEDS OBTAINED, 1928-1931

Cross	Total blossoms	No. of seeds									
		1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9
		Per cent fruitfulness at different seed intervals									
Cox Orange (d) × Baldwin (t)	3719				2.50			14.26			
Cox Orange (d) × Golden Russet (d)	4391				5.41						
Cox Orange (d) × Gravenstein (t)	4550			2.50		10.51		14.44			
Cox Orange (d) × King (t)	5076					14.72					
Cox Orange (d) × McIntosh (d)	3729					1.64					
Cox Orange (d) × Spy (d)	1572										
Cox Orange (d) × Wagener (d)	3817										
Cox Orange (d) selfed	6971										
Golden Russet (d) × Baldwin (t)	3940			2.69		6.07					
Golden Russet (d) × Cox Orange (d)	3787				2.36						
Golden Russet (d) × Gravenstein (t)	3853			2.56							
Golden Russet (d) × King (t)	4103										
Golden Russet (d) × McIntosh (d)	2075			2.02			6.84				
Golden Russet (d) selfed	6627										
Gravenstein (t) × Baldwin (t)	3275	1.58	.52								
Gravenstein (t) × Blenheim (t)	821										
Gravenstein (t) × Cox Orange (d)	4227		15.09								
Gravenstein (t) × Golden Russet (d)	3734			10.39							
Gravenstein (t) × King (t)	4672		1.24								
Gravenstein (t) × McIntosh (d)	3412			9.44							
Gravenstein (t) × Wagener (d)	5586			13.71							
Gravenstein (t) selfed	7908			.87							

TABLE V—*Concluded*

TABLE SHOWING PER CENT FRUITFULNESS OF CROSSES IN RELATION TO THE AVERAGE NUMBER OF SEEDS OBTAINED, 1928-1931

Cross	Total blossoms	No. of seeds									
		1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9
		Per cent fruitfulness at different seed intervals									
King (t) × Baldwin (t)	4205			2.81	3.04	4.05					
King (t) × Blenheim (t)	526					2.80					
King (t) × Cox Orange (d)	4099										
King (t) × Golden Russet (d)	3792			2.89							
King (t) × Gravenstein (t)	3912				4.53						
King (t) × McIntosh (d)	3137					3.98					
King (t) × Wagener (d)	5104			2.59							
King (t) selfed	6359										
Spy (d) × Baldwin (t)	3846				2.60				15.59		
Spy (d) × Ben Davis (d)	4542								12.21		
Spy (d) × Cox Orange (d)	5011								9.72		
Spy (d) × Golden Russet (d)	5113										
Spy (d) × Gravenstein (t)	1323					3.85					
Spy (d) × King (t)	4960				3.15						
Spy (d) × Wagener (d)	305				1.68						
Spy (d) selfed	6250										44.92



To permit of more ready comparison of the results obtained from the different types of crosses in the different series the averages for all three series are summarized in Table IV. This will serve to show the general standing of each with respect to fruit, seed and seedlings. The more detailed tables on which this summary is based are placed in an appendix following this article. Attention is particularly called to Tables XIII, XV, XVIII.

In these tables all percentages are based on the original number of blossoms pollinated, which is the reason that percentages of seeds and seedlings may run over 100%. This practice affords a better standard for the desired comparison than any other that could be devised. The average number of seed per fruit is also given, but this figure is not entirely relied upon because some crosses gave a very small number of fruit, sometimes only one, yet the fruit that is obtained may occasionally show a fairly high seed content. When based on such small numbers, however, the result has little or no meaning. The term "per cent fruit" refers to those apples that remain on after the so-called "June drop."

Stayman Winesap has been excluded from the totals in the diploid  $\times$  diploid class because it does not behave like other varieties of its class, having sticky pollen that agglomerates in bunches and, for this reason, gives poor results in crossing.

Comment on the results as revealed by these tables will be deferred until after the results from other tests are presented.

### **Discussion of Fruitfulness, Seed Content and Seedling Production**

It will be seen from the tables that the general results from Series 1 and Series 2 are strikingly similar in important particulars, although they are based on entirely different varieties. It is apparent at a glance that there is a wide gap dividing the diploid  $\times$  diploid and the triploid  $\times$  diploid crosses on the one hand from the diploid  $\times$  triploid and the triploid  $\times$  triploid on the other. The former represent a highly fruitful type of cross and the latter a very unfruitful one. Whether diploid  $\times$  diploid or triploid  $\times$  diploid crosses come first from the standpoint of fruitfulness depends on the selection of varieties chosen on which to base averages, as there is considerable overlapping in this respect between individual crosses in the two groups. Both diploid and triploid varieties, therefore, are fruitful when crossed by a diploid variety. It is very clearly brought out, however, that, from the standpoint of seed and seedling production, the diploid  $\times$  diploid crosses exceed the triploid  $\times$  diploid by a very wide margin, showing that, with the latter type of cross, a high degree of fruitfulness is possible with a lower seed content than is the case with diploid  $\times$  diploid crosses.

In considering crosses of the unfruitful type, *viz.*, diploid  $\times$  triploid and triploid  $\times$  triploid we find that they give poor results on all counts, with some variation between the different series. From the standpoint of percentage fruit, the triploid  $\times$  triploid type is superior in two out of three

cases and approximately equal in the third case. In several individual cases triploid  $\times$  triploid crosses were commercially fruitful. In both Series 1 and 2 there have been certain inconsistencies, some giving no fruit and others a fair percentage. Boskoop  $\times$  Baldwin, for example, gave 7.76% fruit from 116 pollinations, but Boskoop  $\times$  Blenheim and Boskoop  $\times$  King gave no fruit at all. Baldwin selfed in Series 2 gave 7.67% fruit with 5046 pollinations, but, in this case, the phenomenon of self-compatibility is involved. Whether the high percentage of fruit obtained by using the pollen of triploid varieties on Baldwin is due to selfing can be determined with certainty only by emasculation tests, but later incomplete investigations indicate that this is not the case. From the breeding standpoint triploid  $\times$  triploid crosses are mostly worthless, producing in both Series 1 and 2, even fewer seedlings than in the diploid  $\times$  triploid type. An examination of the detailed figures shows that, in both types there are individual crosses which, though they result in some fruit, produce few fruits with seed and give few or no seedlings, while the seedlings that are produced are of an inferior character or lacking in uniformity. From every point of view, therefore, crosses of these two types are greatly inferior to the other two.

Nevertheless, the comparatively high percentage fruit obtained in certain individual cases of triploid  $\times$  triploid crosses, which has raised the general average for the group, is one that requires explanation. This is particularly noticeable where Baldwin was used in Series 1 (Table XII, p. 328) but, owing to the possibilities of selfing in Series 2, the results from Baldwin, when used as a female parent, have been excluded. Theoretically, this result might be expected as there is a chance for mutual fertilization of diploid or near diploid gametes in triploid  $\times$  triploid crosses.

It is noteworthy that all triploid males when used on Baldwin, with the exception of Blenheim, gave a higher percentage of fruit than was obtained by selfing, in spite of the high degree of self-compatibility exhibited by this variety. The possibility, therefore, that this result may be due to the opportunity offered for mutual fertilization of diploid or near diploid gametes should not be excluded.

The inviability of aneuploid types is indicated by the fact that practically all commercial varieties are either diploids or triploids. Though an excess of forms with 41 chromosomes among triploid seedlings is reported by Moffet (17), the only commercial variety recorded as coming in the group is Wellington Bloomless which, according to Nebel (21) has  $41 + 1$ .

It should be borne in mind when considering these results that incompatibility factors have less effect in triploids than in diploids. Furthermore, when comparing triploid  $\times$  triploid *vs.* triploid  $\times$  diploid we have to deal with a situation different from that which exists when comparing triploid  $\times$  triploid with diploid  $\times$  triploid, since, in unbalanced chromosome types, female gametic fertility is always greater than male.

Considering the evidence gleaned from Series 3 tests and counting only those varieties of known chromosome number, we find, on examining the results from

the triploid  $\times$  triploid crosses, that (1) in amount of fruit produced from a given number of blossoms, (2) in per cent seed and (3) in average number of seeds per fruit, these crosses fall far below the triploid  $\times$  diploid crosses. The fact that the triploid  $\times$  triploid crosses have a small average number of seed and still fewer good seeds, seems to be associated with the unfruitfulness of these crosses. The foregoing results, therefore, strongly corroborate those obtained in the other series. In comparing the results obtained from known diploids with those that may be assumed to be diploids on the basis of their behavior, the similarity is noteworthy.

A study of the data presented in Table V again indicates that seed content must be considered in connection with the chromosome constitution of the varieties concerned. The great difference existing between diploids and triploids as pollinizers is best illustrated in the case of their use on a diploid variety with a comparatively high seed content, like Spy. Here, the fact that all triploid varieties used as male parents gave a low degree of fruitfulness together with a low seed content, and that the reverse held when diploid males were used, is apparent at a glance. The same point may be observed with the other two diploid varieties considered, *viz.*, Golden Russet and Cox Orange. As would be expected, the difference indicated is less apparent when comparatively few-seeded triploid varieties are used as female parents. Nevertheless, even with triploid varieties it will be seen that diploid pollen generally produced a higher seed content, together with a larger number of fruits.

That the degree of fruitfulness is not necessarily in direct proportion to the number of seeds is also apparent. This is particularly true when we compare the few-seeded triploids with the diploids, the former showing a degree of fruitfulness approximately equal to the latter when crossed with a diploid variety, though with only half the seed content. For example, a diploid  $\times$  diploid cross, Spy  $\times$  Golden Russet, gave an average seed content of 9.68 and a percentage fruitfulness of 9.72. On the other hand a triploid  $\times$  diploid cross, Gravenstein  $\times$  Golden Russet, gave an average seed content of 3.29 and a percentage fruitfulness of 10.39. It will also be noted that, in selfing tests, there is considerable inconsistency between seed content and fruitfulness. Thus, the fruit (1.64%) resulting from selfing Cox Orange blossoms gave a seed content falling in the 5-6 interval, while McIntosh and Wagener, with the same seed content gave 10.51 and 14.72% fruitfulness respectively. Thus it will be seen that, in the fruitful types of cross, whether diploid  $\times$  diploid or triploid  $\times$  diploid, we have a higher average seed content than in the unfruitful type, whether triploid  $\times$  triploid or diploid  $\times$  triploid.

Attempts to correlate seed content and fruitfulness more closely result in apparent inconsistencies, which might be expected since, in addition to the group factor of a balanced or unbalanced chromosome constitution which we are here especially considering, there are obviously genetic differences, particularly compatibility and incompatibility factors, which enter into the problem.

### Relation of Seed Content to Weight

As already indicated, many workers have stated that a correlation exists between weight and seed content in the apple. The fact that one-sided apples show some of the carpels empty on the corresponding side is a matter of general observation. Samples picked at random offer little evidence in this connection, since many factors influence size and weight of fruit, and a disturbing factor is introduced in the utilization of fruits resulting from mixed pollination. On the other hand, trees with a very low set due to an unfruitful cross produce few apples, and those that do set may grow abnormally large owing to favorable nutritional conditions. For this reason, it appears desirable that the samples selected should be produced under uniform and normal conditions. In 1931 two varieties, Gravenstein, as representative of a triploid variety with very low seed content and Northern Spy, representative of a diploid variety with an exceptionally high seed content were selected. A tented tree of each variety which had been provided with a hive of bees and an effective pollinizer, Wagener in the case of Gravenstein, Ben Davis in the case of Spy was used. All the apples on each tree were taken, 500 in the case of Gravenstein and 1,596 in the case of Spy. By thus providing optimum conditions for pollination we naturally reduced the production of abnormal apples likely to result from imperfect fertilization, which undoubtedly affected the results, but gave a value for the effect of seed content.

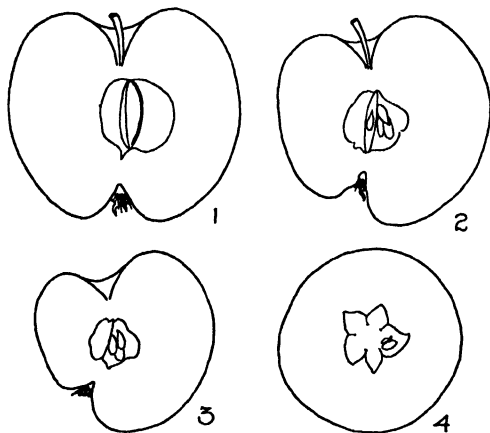
The coefficient of correlation using all seeds, whether filled or not, was, in the case of Gravenstein  $.055 \pm .0366$ , which is not significant, and for Spy,  $.3467 \pm .0148$ , which is statistically just significant. Since results with Gravenstein are not in line with those obtained by Einset (6) and since the correlation in the case of Spy is not as great nor as striking as might have been expected, it was decided to duplicate the work with Gravenstein and Spy and, in addition, to run similar correlations with King pollinated with Wagener and Baldwin pollinated with Cox Orange. The following number of fruits were examined: Gravenstein, 1,100; Spy, 1,000; King, 314; and Baldwin, 1,000. The coefficients of correlation obtained in 1932 were as follows: Gravenstein,  $.0025 \pm .0302$ ; King,  $.1103 \pm .0557$ ; Baldwin,  $.2723 \pm .0293$ ; Spy,  $.3069 \pm .0286$ . In the Gravenstein, King and Baldwin varieties, no significant correlation was obtained, but in the case of the Spy the result may be considered just significant. Since the first three of these are triploids and the last a diploid it was thought that possibly the existence or otherwise of a correlation might depend on chromosome constitution. Accordingly another diploid, *viz.*—Wagener, was selected and one thousand apples from an open pollinated lot used for the purpose of our calculations. A correlation of  $.07304 \pm .0315$  was obtained, which is not significant.

The foregoing data show that there is no definite correlation between the weight and the number of seeds per apple in the Gravenstein, King, Wagener and Baldwin varieties under the conditions tested. In the Spy variety a correlation just statistically significant was obtained, but even this cannot be considered at all marked.

## Morphological Abnormalities

### 1. FRUIT DISTORTION

It has already been shown that apples coming off in that wave of abscission to which the name "June drop" is applied have consistently produced a lower average seed content than those that remained on the tree. The relative uniformity in size and weight prevailing among the individual apples



FRUIT DISTORTION IN BEN DAVIS

FIG. 1. Longitudinal section of normal apple containing 15 seeds, viz., 2 carpels with 4 seeds and 2 carpels with 3 seeds.

FIG. 2. Longitudinal section of apple with 3 carpels containing 3 seeds each, the remainder empty.

FIG. 3. Longitudinal section of apple with 3 seeds in one carpel, the remainder empty.

FIG. 4. Cross section of same apple. Note lack of reduction in transverse diameter.

distortion is frequently observed and even seedless apples of almost cylindrical shape may be found.

A study of one-sided specimens among Spies revealed an interesting situation. There does not appear to be any reduction in the transverse diameter due to reduced seed content, but there occurs a marked depression of the axial diameter of the fruit on the side where there are no seeds. Out of the one thousand Spies examined 9.30% were thus "drawn" at the calyx-end, giving the one-sided effect. By dividing the apples into two groups, (1) normal and (2) malformed, one hundred in each group, it was found that the average seed content of the first group was 9.74 and of the second 6.05. Further studies on open-pollinated Black Ben Davis gave the results shown in Table VI.

on limb-units or even whole trees pollinated with a cross-fruitful variety, has been marked. It has also been noted that, in certain varieties, one-sided apples result from the failure of the seeds on one side to develop. This type of distortion appears to be most pronounced in apples with a relatively high seed content and hence it would be expected that such apples would more commonly occur among the many-seeded diploid variety than among the few-seeded triploid varieties.

In such varieties as Spy and Deacon Jones, in which the typical seed content is twenty, distortion of fruit containing less than the normal number of seeds is common. In the case of Spy such fruit is of the characteristic one-sided type. In Deacon Jones a similar

TABLE VI

THE RELATION OF SEED CONTENT TO ONE-SIDED FRUIT IN BLACK BEN DAVIS

Kind of fruit	No. examined	Av. seed content
Normal fruit	103	6.13
Malformed fruit	85	4.54

A similar classification of Ben Davis, also from open-pollinated trees, gave the results shown in Table VII.

It is unfortunate that conditions did not permit the securing of further data on this point, but it is only to be expected that varieties in which malformed apples are common would show an apparent correlation between seed content and weight, while those that do not respond in this manner to

TABLE VII  
THE RELATION OF SEED CONTENT TO ONE-SIDED FRUIT  
IN BEN DAVIS

Kind of fruit	No. examined	Av. seed content	Av. no. empty carpels
Normal	94	6.67	0.340
Strongly malformed	79	3.61	2.00
Slightly malformed	30	4.47	1.67

reduced seed content and weight, would show no such correlation. It would also be expected that more distortion would occur under conditions of insufficient pollination than would be the case where conditions for adequate pollination were provided, as with the tented trees. Hence, by selecting only fruit from well-pollinated trees, we secure lower values than would otherwise be the case.

Among varieties in which one-sided or distorted apples were not found in these studies were Gravenstein, King, Baldwin, Wagener and Blenheim. It is of interest to note that in the first four of these the writers were unable to demonstrate any significant correlation between seed content and weight. Regarding the fifth variety they have no information on this point.

## 2. PREMATURE DROP AND OPEN CALYX-END

### (a) Preliminary Studies

In one of the varieties mentioned, *viz.*, Gravenstein, in which no correlation between seed content and weight was demonstrated and in which no distortion of the fruit due to failure of seeds to develop has been found, data bearing on the influence of imperfect pollination and consequent low seed content are available. It should be noted that, in this variety, the average seed content is lowest of all varieties studied. In addition, the phenomenon of self-compatibility is marked, comparing with Spy in this respect. It has long been noted in Gravenstein that there occurs, in certain seasons, a later drop of practically fully grown apples that ripen prematurely and drop in late August and early September. This condition was very pronounced in the season of 1928 and it was noted that such apples also had a lower seed content than apples remaining on the tree. In 1932 more definite evidence was obtained that appeared to connect premature drop with imperfect pollination and low seed content. This evidence was obtained from a series of Gravenstein trees tented during bloom, some of which were (1) supplied with bees to serve as pollinators, and with a source of foreign pollen in the form of a "bouquet" of blossoming limbs of the desired variety, (2) without bees, and (3) with neither bees nor bouquets, as indicated later. The results were compared

with those from an open-pollinated untented tree. While this tree received a measure of effective pollination, it is only reasonable to suppose that the pollination was not as complete as with tented trees. Though based only on a single season's observations, the results appear sufficiently suggestive to justify consideration at this point. The need of much further work in order definitely to settle the different issues raised is clearly indicated.

The first fact noted in connection with these tests is that a comparatively large number of drops were present under the open-pollinated tree, whereas the Wagener-pollinated tree showed very few drops. So little fruit was obtained from the selfed or ineffectively pollinated trees, that the results from such trees were not significant.

Examination of the drop fruit from the two trees referred to showed that not only were the drop apples less numerous beneath the Wagener-pollinated tree, but that the content of developed seeds was greater and that of undeveloped seeds, represented by fragments of undeveloped embryos, less than those beneath the open pollinated tree.

The detailed results of the examination of these drops are shown in Table VIII.

TABLE VIII

THE RELATION OF SEED CONTENT TO PREMATURE DROP OF GRAVENSTEIN

Treatment	Number drops	Av. no. seeds	Av. no. undeveloped seeds
Bees and Wagener bouquets	134	5.24	2.28
Open-pollinated	289	3.67	3.29

On cutting open the apples to make the seed count it was noted that the structure of many of the drop apples was abnormal, in that the calyx-end was not closed in the ordinary way. The cavity, sometimes erroneously called the "calyx tube" in such apples, extended downward penetrating the core lines and resulted in an open-core condition.

This open-core condition had evidently exposed the core region to the invasion of certain saprophytic organisms resulting in the condition known as "moldy core." A striking difference between the incidence of moldy core in the two series was at once apparent, *viz.*, 5.22% from the Wagener-pollinated and 35.29% from the open-pollinated. An accurate count showing the percentage of open blossom end present in the two sets of drops is not available.

In view of the foregoing results it was decided to examine the apples that still remained on the trees throughout the entire series in order to determine: (1) whether a definite relation existed between seed content and the open-core condition on an open-pollinated tree, where a measure of effective pollination had been obtained, and (2) to study the effect of different degrees of effectiveness of pollination on the same condition.

#### (b) Procedure and Results

In connection with the first study individual apples were cut from an open-pollinated tree and classified as to seed content and open or closed calyx.

For the second study fruit from each of the six tented trees was saved and similarly classified. In the case of the trees that yielded small crops due to lack of suitable pollinizers or absence of pollinators, the entire crop was harvested and examined. From the remaining trees (*i.e.*, the open-pollinated and that supplied with bees and Wager bouquets) sufficient apples were removed to ensure a satisfactory average.

The results from an examination of 1200 apples taken from the open-pollinated tree are classified in the accompanying table with respect to total number and percentage of fruits affected with "moldy core," together with the average seed content for the different groups.

The foregoing results show clearly that the open blossom-end condition is associated with "moldy core." The difference in the average seed content between fruits with open and closed calyx-end does not seem great, but it evidently is great enough to be highly significant as indicated in the results from an examination of the fruit from the entire tented series. This is indicated in the next table.

TABLE IX  
THE RELATION BETWEEN MOLDY CORE AND OPEN CALYX-END

Condition	No. fruit	Av. seeds	% fruit
Non-moldy and closed	997	3.54	83.08
Moldy and open	187	3.24	15.58
Moldy and closed	13	3.08	1.08
Non-moldy and open	3	3.00	0.25
	1200		

TABLE X  
SEED CONTENT, OPEN CALYX-END AND MOLDY CORE UNDER DIFFERENT CONDITIONS OF CONTROLLED POLLINATION

Treatment	No. fruit	Av. seeds per apple	% seeds	Av. undeveloped seeds per apple*	% calyx open	% moldy core
No bees and no bouquets	28	1.14	47.06	1.29	57.14	71.43
Bees and no bouquets	194	1.46	22.29	5.10	45.88	57.73
Bees and Blenheim (triploid) bouquets	116	1.27	16.84	6.26	37.93	37.07
No bees and Wager (diploid) bouquets**	435	2.30	28.71	5.71	26.90	24.14
Open pollinated	1200	3.49	50.11	3.47	15.83	16.67
Bees and Wager (diploid) bouquets	1100	4.73	62.89	2.79	0.82	1.00

\*Undeveloped seeds were those that were visible as very minute brown specks, representing seeds that had not developed in normal manner.

\*\*Pollination was effected by a blast of air from an orchard duster blowing through the bouquets and over the tree.

### (c) Discussion

The association between the degree of pollination, seed content, the amount of open calyx-end and of moldy core appears evident from a study of these figures. To emphasize the close relation between seed content and open



calyx-end, the fruit has again been classified according to seed content, regardless of its origin, and the results presented in the next table. Four intervals are given, *viz.*, (1) over one but less than two, (2) over two but less than three, (3) over three but less than four, and (4) over four.

TABLE XI  
RELATIONSHIP OF AVERAGE SEED CONTENT TO OPEN CALYX

Good seeds	Av. no. seeds	Av. no. of undeveloped seeds	No. of fruit examined	% with open calyx-end
Over 1	1.38	5.18	338	44.08
Over 2	2.30	5.71	435	26.90
Over 3	3.49	3.47	1200	15.83
Over 4	4.73	2.79	1100	.82,

The conclusion that an increasing seed content is associated with a decreasing percentage of fruit with an open calyx-end would appear justified from the results. There is also a correlation between the number of undeveloped or aborted seeds and closure of the calyx-end, but, in this case a large number of aborted seeds is associated with a high percentage of apples with the calyx-end open and *vice versa*. In the average number of undeveloped seeds there is one apparent contradiction. The number is larger in the group "over 2 seeds" than in the group, "over 1 seed." This may be explained by referring to Table XI. Where no bees or no bouquets were used, in addition to obtaining few seeds in the apples, very few aborted seeds were produced. This materially lowers the average number of aborted seeds for this group.

The effect of the degree of pollination that takes place is apparent from these figures. Thus, where bees and an effective pollinizer, namely, Wagener, were used, 4.23 seeds per apple were produced and only .82% of the fruits had an open calyx-end. When an ineffective pollinizer, Blenheim, was used, the number of seeds per apple was reduced to 1.27 and the percentage of fruit with open calyx-end increased to 37.93%. The results from the open pollinated tree, which was, however, surrounded with both effective and ineffective pollinizers, were intermediate.

These results, based on one year's figures, very clearly indicate that there is a definite relation between the effectiveness of pollination and the closure of the calyx-end. The fact that this "calyx tube" does not close exposes the seed cavity to infection from various organisms, and this would appear to be the true cause of moldy core, the associated organisms being secondary.

The fact that the condition was particularly severe in 1932, when weather conditions during blossoming were unusually unfavorable for pollination, is of interest. No attempt was made to make any further studies of moldy core, nor of the organisms associated therewith. Nor has a study of the development of the apples resulting in open or closed calyx-end been at-

tempted. This would require a complete study of the development of the fruit from the time of fertilization onward. However, a study of the calyx-end or "eye" of the apple has been recently made by Tetley (22) in connection with disease known as "eye-rot" caused by *Nectria galligena*. She notes that the tube formed by the style may, in the Bramley seedlings, be open all the way down into the core exactly as has been noted in the case of Gravenstein. Usually, however, the way is blocked owing to the interlocking and growing together of some of the surface cells. It is pointed out that in very young apples before they have set, the calyx cavity is shallow and open, since the sepals project horizontally from the apex of the apple. When the apple has set, the rapid growth of the fruit brings the base of the sepals into a horizontal position, thus closing the cavity to a very large extent.

No extensive survey has been made to determine the occurrence of this condition in apple varieties, but in addition to Gravenstein and Bramley seedling, we have noted it also in another triploid variety, *viz.*, Boskoop. This is of interest in view of the observed fact that, in triploids, abnormalities are likely to develop late in the life cycle. The evidence presented above is not sufficient to permit of too definite conclusions, nor is it possible to state that no other factors, besides those indicated, have a bearing on the problem.

### Summary

1. Results obtained from apple crosses in which the limb unit method was employed and a large number of pollinations performed without emasculation, gave sufficiently comparable results to those obtained with fewer numbers with emasculation, to afford useful corroborative evidence as to the behavior of the different types of crosses.

2. Large counts of seed content from drop and picked apples show a consistently larger average number in the latter, indicating the importance of seed content in fruit setting.

3. From the standpoint of fruitfulness, both diploids and triploids as female parents are fruitful when crossed with diploid varieties.

4. Both diploids and triploids, as female parents, are inferior in fruitfulness when crossed with triploid varieties, considerable overlapping again occurring between individual crosses, particularly in the triploid  $\times$  triploid group, some of which were fairly fruitful. The diploid  $\times$  triploid cross is uniformly unfruitful.

5. Self-compatibility is a varietal characteristic. The relatively high self-compatibility of Baldwin, as evidenced by percentage fruit, seed and seedlings produced, is particularly noticeable.

6. Diploid varieties, as female parents, have consistently given a higher seed content than triploid varieties and, within the variety, the seed content is affected by the chromosome constitution of the male parent, diploids as male parents giving a higher seed content than triploids.

7. Though diploid  $\times$  diploid and triploid  $\times$  diploid crosses are on the average of approximately equal fruitfulness, the latter have a much lower average seed content. The percentage fruitfulness is not necessarily directly proportional to the seed content, as is explained more fully elsewhere.

8. Though triploid  $\times$  triploid crosses may sometimes exceed the diploid  $\times$  diploid type in fruitfulness, the latter have a higher average seed content.

9. As might be expected, the results obtained from seedlings are in general agreement with those based on seed alone, and the order of seedling production is the same in all tests, as follows: first, diploid  $\times$  diploid; second, triploid  $\times$  diploid; third, diploid  $\times$  triploid; and fourth, triploid  $\times$  triploid.

10. Data not included in the foregoing paper indicate that, whereas the male parent has no measurable effect on germination, triploids as a group show a much lower germination than diploids when used as females.

11. Tests conducted in 1931 showed no correlation between seed content and weight in the case of Gravenstein, and a barely significant correlation in the case of Spy, results which were confirmed in 1932. Further tests conducted with Wagener, Baldwin and King in 1932 gave no significant correlation with those varieties.

12. In apple varieties with a normally high seed content, failure of seed to develop on one side of an apple results in distorted or "one-sided" fruit. In certain varieties also, seedless or nearly seedless apples have been found in which the distortion extends to both sides and the fruit is almost cylindrical in shape.

13. Examination of "one-sided" and normal apples from open pollinated trees revealed the fact that, in all cases, the average number of seeds was lower and the average number of empty carpels was higher in the former group.

14. Imperfect pollination, low seed content, open calyx-end and "moldy core," appear, on the basis of one season's observations, to be associated phenomena in Gravensteins, but further work is necessary before definite conclusions are possible.

### Appendix

The following are the detailed tables upon which the foregoing discussion has been largely based. Attention is particularly called to the Summary Tables XIII, XV, and the last three lines of Table XVIII.



FIG. 1 General view of seedlings



FIG. 2 29 Cox Orange  $\times$  G Russet (Diploid  $\times$  diploid) 33 King  $\times$  G Russet (Triploid  $\times$  diploid) 34 Spn  $\times$  Cox Orange (Diploid  $\times$  diploid)



FIG. 3. 71. Cox Orange (Diploid) selfed



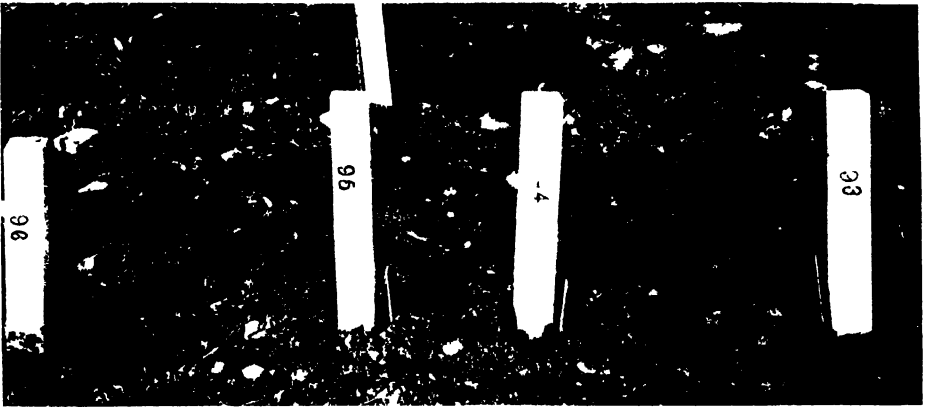


FIG. 4. 93. *Russet*  $\times$  *Gravenstein* (Diploid  $\times$  triploid). 94. *Blenheim*  $\times$  *Gravenstein* (Triploid  $\times$  triploid). 95. *McIntosh*  $\times$  *Gravenstein* (Diploid  $\times$  triploid). 96. *Baldwin*  $\times$  *Gravenstein* (Triploid  $\times$  triploid).



FIG. 5. 83. *Stark*  $\times$  *Fallawater* (Triploid  $\times$  triploid). 84. *Blenheim*  $\times$  *Duchess* (Triploid  $\times$  triploid). 85. *Blenheim*  $\times$  *Wagner* (Triploid  $\times$  diploid). 86. *Stark*  $\times$  *Fameuse* (Triploid  $\times$  diploid). 87. *Blenheim*  $\times$  *Fallawater* (Triploid  $\times$  triploid).



FIG. 6. 21. *Baldwin* (Triploid) selfed.



TABLE XII

RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: diploid × diploid					
Delicious × Ben Davis	48	37.50	289.58	7.72	185.42
Delicious × Cox Orange	57	24.56	154.39	6.27	122.81
Delicious × Duchess of Oldenberg	53	13.21	107.77	7.85	81.13
Delicious × Golden Russet	64	21.87	184.38	8.43	142.19
Delicious × McIntosh	46	15.22	108.69	7.14	71.74
Delicious × Rome Beauty	43	11.63	97.67	8.40	5.17
Delicious × Northern Spy	61	22.95	157.38	6.86	122.95
Delicious × Stayman Winesap	47	—	—	—	—
Ben Davis × Delicious	28	14.28	114.29	8.00	92.86
Rome Beauty × Delicious	16	31.25	112.50	3.60	75.00
Cox Orange × Delicious	22	27.27	219.18	8.00	161.54
McIntosh × Delicious	8	25.00	137.50	5.50	12.50
Red Rome Beauty × Cox Orange	4	25.00	200.00	8.00	175.00
Red Rome Beauty × S. Winesap	7	—	—	—	0.00
Red Spy × Red Rome Beauty	49	44.90	387.75	8.59	198.78
Red Spy × Cox Orange	41	21.95	197.56	9.00	102.44
Red Spy × McIntosh	28	14.25	96.43	6.75	21.43
Red Spy × Stayman Winesap	10	—	—	—	0.00

Type of cross: diploid × triploid

Delicious × Baldwin	71	1.41	8.45	6.00	8.45
Delicious × Crimson Bramley	39	—	—	—	—
Delicious × Gravenstein	59	3.42	23.73	7.00	8.47
Delicious × King	81	—	—	—	—
Delicious × Ribston	50	—	—	—	—
Delicious × Stark*	68	—	—	—	—
Delicious × Nonpareil*	74	6.75	42.02	6.20	28.38
Deacon Jones × Gravenstein	42	—	—	—	—
Deacon Jones × Crimson Bramley	33	—	—	—	—
Deacon Jones × Baldwin	28	—	—	—	—
Deacon Jones × Ribston	19	—	—	—	—
Deacon Jones × Blenheim	22	—	—	—	—
Deacon Jones × Stark*	28	3.57	7.14	2.00	3.57

\*Assumed to be triploids on account of their genetic behavior; later determined as such by Dr. M. V. Roscoe.



TABLE XII—Continued  
RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: triploid × diploid					
Boskoop × Red Astrachan	96	12.50	38.54	3.08	15.63
Boskoop × Ben Davis	89	29.21	84.26	2.88	1.12
Boskoop × Cox Orange	61	24.59	98.36	4.00	39.34
Boskoop × Delicious	122	28.70	77.87	2.71	16.39
Boskoop × Duchess of Oldenberg	104	40.38	149.03	3.69	40.38
Boskoop × Golden Russet	112	39.29	132.14	3.36	28.57
Boskoop × McIntosh	89	13.48	30.34	2.25	5.62
Boskoop × Red Rome Beauty	102	27.55	96.08	3.50	14.71
Boskoop × Northern Spy	118	35.60	119.49	3.35	25.42
Boskoop × Stayman Winesap	97	20.60	59.79	2.90	10.31
Ribston × Delicious	16	31.25	112.50	3.60	12.50
Baldwin × Delicious	11	63.63	390.90	6.14	63.64
Crimson Bramley × Delicious	9	44.44	33.33	.75	11.11
Type of cross: triploid × triploid					
Boskoop × Baldwin	116	7.76	15.52	2.00	2.59
Boskoop × Blenheim	78	—	—	—	—
Boskoop × King	92	—	—	—	—
Boskoop × Stark	103	1.94	4.85	2.50	0.00
Boskoop selfed	62	3.22	11.29	3.50	0.00
Type of cross: triploid × assumed diploids					
Boskoop × Adams Pearmain	118	21.18	106.70	5.04	22.88
Boskoop × Deacon Jones*	143	15.38	58.04	3.27	15.38
Boskoop × Grimes Golden*	96	38.54	123.95	3.21	33.33
Boskoop × Opalescent	112	33.03	111.61	3.38	45.75
Boskoop × Yellow Transparent	113	17.70	51.33	2.90	19.47
Type of cross: diploid × assumed diploids					
Delicious × A. Pearmain	62	6.45	40.32	6.25	29.03
Delicious × Deacon Jones†	86	38.37	261.62	6.82	194.19
Delicious × Golden Russet†	67	23.38	106.53	7.27	114.93
Delicious × Jonathan†	58	15.52	110.34	7.11	75.86
Delicious × Yellow Transparent	56	50.00	303.57	7.39	205.36
Ben Davis × Deacon Jones†	36	16.66	88.82	5.33	63.89
Rome Beauty × Deacon Jones†	13	69.23	146.15	2.11	76.92
R. Rome Beauty × Deacon Jones†	7	28.57	200.00	7.00	128.57
R. Rome Beauty × York Imperial	4	75.00	700.00	9.33	200.00
Red Spy × Deacon Jones†	15	13.33	140.00	10.50	73.33
Red Spy × Jonathan†	22	27.27	195.45	7.16	104.55
Red Spy × York Imperial	20	25.00	190.00	7.60	115.00

\*Since writing the foregoing the chromosome number of these varieties has been determined as 34 by Dr. M. V. Roscoe.

†Since writing the foregoing these varieties have been shown to be diploids by Dr. M. V. Roscoe.

TABLE XII—Continued  
RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: assumed diploid × diploid					
Deacon Jones × Red Astrachan	47	19.05	129.78	6.77	78.72
Deacon Jones × Ben Davis	28	21.43	128.57	6.00	67.86
Deacon Jones × Delicious	45	28.89	224.44	7.77	117.78
Deacon Jones × Duchess of Oldenberg	28	10.71	78.57	7.33	53.57
Deacon Jones × McIntosh	26	5.27	5.27	1.00	5.27
Deacon Jones × Yellow Bell-flower	22	13.64	100.00	7.33	63.64
Deacon Jones × Stayman Winesap	29	3.45	3.48	1.00	0.00

TABLE XIII  
SUMMARY OF RESULTS FROM DIFFERENT TYPES OF CROSSES, 1930-1931 (Series 1)

Diploid × diploid	568	23.24	176.41	7.59	115.85
Diploid × triploid	444	0.68	4.50	6.67	2.70
Triploid × diploid	1026	28.46	93.37	3.28	20.86
Triploid × triploid	286	3.15	6.29	2.00	1.05
Assumed diploid × diploid	225	16.00	108.44	6.78	61.72
Diploid × diploid (without Stayman Winesap)	196	17.86	123.98	6.94	70.92
Diploid × assumed diploid	446	26.23	176.68	6.74	118.39
Diploid × triploid (with Stark and Nonpareil)	614	1.47	9.28	6.33	5.37
Triploid × assumed diploid	582	24.26	87.80	3.62	26.12
Triploid × triploid (with Stark)	389	2.83	5.91	2.09	0.77
Triploid selfed	62	3.22	11.29	3.50	—
Diploid selfed	67	1.49	11.94	8.00	2.99

TABLE XIV  
PER CENT FRUIT, SEEDS, AND SEEDLINGS PRODUCED FROM VARIOUS APPLE CROSSES, SERIES 2, 1930-1931

Cross	Number blossoms pollinated	Av. % fruit after July drop	Av. no. seeds per fruit	% seeds 1930-31	Av. % seedlings 1930 and 1931
Diploid × diploid					
Cox Orange × Golden Russet	3207	15.47	6.79	100.31	49.83
Cox Orange × McIntosh	3298	9.43	6.47	57.10	29.78
Cox Orange × Wagener	3258	13.35	6.23	75.38	28.97
Cox Orange selfed	5018	1.79	5.22	9.05	3.51
Spy × Ben Davis	4542	15.59	8.70	114.88	63.41
Spy × Cox Orange	3674	10.23	8.86	76.24	49.80
Spy × Golden Russet	3551	10.59	9.61	88.26	48.27
Spy selfed	4058	1.89	4.66	6.09	3.95
Golden Russet × Cox Orange	2602	6.53	6.54	32.67	17.10
Golden Russet × McIntosh	2075	6.84	6.27	35.04	13.78
Golden Russet selfed	3892	0.90	2.86	2.06	1.28

TABLE XIV—*Continued*

PER CENT FRUIT, SEEDS, AND SEEDLINGS PRODUCED FROM VARIOUS APPLE CROSSES, SERIES 2, 1930-1931

Cross	Number blossoms pollinated	Av. % fruit after July drop	Av. no. seeds per fruit	% seeds 1930-31	Av. % seedlings 1930 and 1931
Diploid × triploid					
Cox Orange × Baldwin	2420	3 24	4 68	13 35	5 21
Cox Orange × Gravenstein	3283	5 88	4 84	25 84	13 80
Cox Orange × King	3177	1 92	3 85	6 55	3 02
Spy × Baldwin	2649	2 15	4 38	7 93	5 02
Spy × King	3227	1 95	3 76	5 83	3 35
Golden Russet × Gravenstein	2379	2 69	4 13	8 15	3 54
Golden Russet × King	2581	2 63	3 61	6 86	2 36
Golden Russet × Baldwin	2465	2 31	3 33	7 01	3 49

Triploid × diploid					
Gravenstein × Cox Orange	2776	16 57	2 93	42 36	9 04
Gravenstein × Golden Russet	2753	11 12	3 00	26 63	4 32
Gravenstein × McIntosh	2064	13 08	3 35	29 36	5 72
Gravenstein × Wagener	4175	15 38	3 66	46 11	14 35
King × Cox Orange	2967	2 83*	5 46	13 25	2 26
King × Golden Russet	2702	3 00	5 08	12 40	4 15
King × McIntosh	2755	4 21	4 19	15 06	3 27
King × Wagener	3496	3 15	5 39	15 10	1 43
Baldwin × Cox Orange	3949	11 47	4 93	48 67	17 30
Baldwin × Golden Russet	3307	9 13	4 31	35 56	7 14
Baldwin × Spy	3575	9 87	5 38	46 24	12 67

\*This is lower than the average figure which is 5.08% for the period 1928-1932 inclusive.

Triploid × triploid					
Gravenstein × Baldwin	2451	0 57	2 80	1 14	0 20
Gravenstein × King	3159	1 08	2 58	1 96	0 32
Gravenstein × Blenheim	162	3 70	1 60	4 94	1.23
Gravenstein selfed	5581	1 00	3 38	2 85	0.20
King × Baldwin	3008	3 59	3 19	9 54	1.50
King × Gravenstein	2621	3 40	3 75	10 87	1.41
King × Blenheim	134	0 00	0 00	0 00	0 00
King selfed	5106	2 82	2 88	6 54	0 74
Baldwin × Gravenstein	3227	9 30	3 53	30 77	7.91
Baldwin × King	3066	7 66	3 41	24 69	5 97
Baldwin × Nonpareil	139	8 63	2 09	16 55	4.32
Baldwin × R. I. Greening	458	6 33	2 96	14 85	1.09
Baldwin selfed	5046	7 67	3.19	20.21	5 47

TABLE XV

SUMMARY OF RESULTS FROM DIFFERENT TYPES OF CROSSES, 1928-1932 (Series 2)

Type of cross	Total blossoms	% fruit	% seeds*	Av. no. seeds per fruit*	% viable seedlings 1930-31
Diploid × diploid	43,294	12.11	75.70	7.07	38.52
Diploid × triploid	46,426	2.89	9.93	4.04	5.17
Triploid × diploid	60,284	10.46	36.79	4.41	8.29
Triploid × triploid (excluding Baldwin as female parent)	21,645	2.72	6.81	3.39	0.83
Triploid × triploid	32,267	4.71	13.49	3.47	2.91
Diploid selfed	22,894	1.66	6.38	4.36	2.98
Triploid selfed	22,021	3.56	9.04	3.11	2.07

\*Seed counts made on basis of actual number of fruit harvested.

TABLE XVI

TABLE SHOWING STANDING OF DIFFERENT TYPES OF CROSSES IN SERIES 2 FOR FIVE YEARS

Type of cross	% fruit	Av. seeds per fruit	% seeds	Type of cross	% fruit	Av. seeds per fruit	% seeds
Diploid × diploid	12.11	7.07	75.70	Triploid × diploid	10.46	4.41	36.79
Diploid × triploid	2.89	4.04	9.93	Triploid (excluding Baldwin) × triploid	2.72	3.39	6.81

TABLE XVII

TABLE SHOWING STANDING OF DIFFERENT TYPES OF CROSSES FOR SERIES 1 AND 2 FOR TWO YEARS

Type of cross	% fruit		Av. seeds per fruit		% seeds		% seedlings	
	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
Diploid × diploid	23.24	11.50	7.59	7.60	176.41	77.41	115.85	38.52
Diploid × triploid	0.68	1.56	6.67	4.20	4.50	6.02	2.70	5.17
Triploid × diploid	28.46	9.45	3.28	4.08	93.37	10.51	20.86	8.29
Triploid × triploid (excluding Baldwin)	3.15	2.10	2.00	3.27	6.29	5.62	1.05	0.83

TABLE XVIII  
RESULTS IN FRUIT AND SEED PRODUCTION OF CERTAIN CROSSES WITH BLENHEIM AND GRAVENSTEIN, 1929 (Series 3).

Cross	Number blossoms pollinated	Total no. of fruit	Total no. of seeds			Av. no. seeds per fruit	Av. good seeds per fruit	%	% fruit
			Good seeds	Poor seeds	Total seeds				
Blenheim × Delicious (?)	73	10	18	40	58	31	1.80	24.66	15.07
Blenheim × Duchess (?)	148	20	54	29	83	85	2.70	36.49	13.51
Blenheim × Jonathan (A)	160	8	33	12	45	22	4.13	20.63	6.88
Blenheim × McIntosh (d)	90	13	27	31	58	27	2.08	30.00	16.67
Blenheim × Nonpareil (t)	199	1	3	0	3	5	3.00	1.51	0.52
(Roxbury Russet)									
Blenheim × Red Astrachan (d)	203	21	59	41	100	73	2.81	29.06	10.84
Blenheim × Ribston (t)	107	0	0	0	0	0	0	0	0
Blenheim × Rome Beauty (d)	127	13	50	19	69	43	3.85	39.37	10.24
Blenheim × Wagener (A)	172	15	69	30	99	29	4.60	40.12	9.30
Blenheim × Wealthy (d)	181	18	45	32	77	61	4.28	24.86	10.50
Blenheim × Wolf River (A)	149	18	56	62	118	55	3.11	37.58	14.77
Blenheim × Yellow Transparent (X)	204	15	54	36	90	35	3.60	26.47	7.35
Blenheim × York Imperial (?)	112	14	56	29	85	29	4.00	50.00	13.39
Gravenstein × Baldwin (t)	824	3	4	3	7	25	1.33	0.49	0.36
Gravenstein × Blenheim (t)	—	4	7	2	9	40	2.25	No record	
Gravenstein × Cox Orange (d)	1040	56	133	95	228	328	2.38	12.79	6.35
Gravenstein × Duchess (?)	428	16	27	43	70	102	1.69	6.31	3.97
Gravenstein × Golden Russet (d)	981	75	179	137	316	507	2.39	18.25	8.36
Gravenstein × Gravenstein (t)	2148	11	10	16	26	106	0.91	0.47	0.61
Gravenstein × King (t)	1079	3	3	1	4	35	1.00	0.28	0.28
Gravenstein × McIntosh (d)	340	42	66	122	188	250	1.57	19.41	4.67
Gravenstein × Red Astrachan (d)	398	23	60	58	118	144	5.13	15.08	5.78
Gravenstein × Spy (d)	288	10	28	22	50	59	5.00	9.72	3.82
Gravenstein × Stark (t)	1098	52	142	151	293	279	5.63	12.93	5.19
Gravenstein × Wagener (X)	745	120	375	243	618	611	5.15	50.34	6.04
Gravenstein × Wolf River (X)	843	39	95	99	194	219	4.97	11.27	5.58
Gravenstein × Yellow Transparent (?)	1158	49	128	135	263	279	2.61	11.05	4.66
Triploid × known triploid	4357	18	20	20	40	171	0.61	0.46	0.41
Triploid × all others (X)	8948	647	1754	1425	3179	3268	2.71	19.60	7.23
Triploid × known diploid	3658	271	647	557	1204	1492	2.39	17.69	7.41

(t) represents triploids; (d) diploids; (?) unknown; X, classified as unknown at time of writing, but since shown to be diploids; A, probably diploid. Crosses with known diploids are given for comparison.

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# A STUDY IN THE RELATIVE CONSTANCY OF HIVE BEES AND WILD BEES IN POLLEN GATHERING<sup>1</sup>

BY W. H. BRITTAIN<sup>2</sup> AND DOROTHY E. NEWTON<sup>3</sup>

## Abstract

The chief purpose of the investigations described in this paper was to determine the relative pollen constancy of the various insect pollinators of the apple. In addition to the hive bee, these proved to be solitary bees belonging to the genera *Halictus* and *Andrena*, with *Bremidae* and various *Diptera* playing a minor role. It is pointed out that apparent flower constancy depends a great deal upon availability and that almost any result may be obtained by choosing certain periods in which to make tests. The results, based on analyses of the pollen loads of bees captured in apple blossoms, place the hive bee first as regards the number of pure loads, followed by *Halictus*, with *Andrena* a poor third. Taking into consideration the results of the entire season, and the analyses of bees from different sources of pollen, *Halictus* came first in these particular tests, but the difference is not considered significant. The supposed superiority of the hive bee from the standpoint of constancy does not appear to have been proved. Both *Halictus* and the hive bee, however, evidenced a significant superiority over the *Andrena* species studied.

## Review of the Literature

Aristotle is authority for the statement that, "On each trip the bee does not fly from the flower of one species to that of another, but goes, for example, from violet to violet, without touching any other flower before returning to the hive." Darwin (10) also believed that bees exercised discrimination in their visits, the nectar gatherers choosing bloom that gives the most nectar in the easiest way and the pollen gatherers similarly, in the case of pollen. He considers both hive and bumble bees to be good botanists, recognizing varieties of the same species even though of different color. He does not consider the habit of constancy to be invariable, however, especially when only a few plants of the same species grow together. He emphasizes the importance to the plant of the habit of constancy in helping to ensure cross-pollination.

Since Darwin's time a great deal of evidence, much of it conflicting, has been brought forward regarding constancy in bees. Among these contributions may be mentioned those of Bennett (1, 2, 3, 4), Christy (9), Mueller (15), Bulman (7), Ord (16), Plateau (20), Pérez (18), Wagner (23), Lovell (14), Robertson (21), and Kranichfeld (13). Most of these papers and others not mentioned have been carefully reviewed by Clements and Long (8) and need not be repeated. The work of Bonnier (6) on the division of labor among bees, however, deserves mention. This worker, by a system of marking and subsequent observations of the marked bees, claims to have demon-

<sup>1</sup> Manuscript received July 6, 1933.

Part of this investigation was carried on at Macdonald College, Quebec, through funds supplied by that institution, and the remainder at various points in Kings County, Nova Scotia, as a part of the project in apple pollination carried out under the direction of a committee of the Dominion Department of Agriculture, of which Mr. Arthur Gibson, Dominion Entomologist, was chairman.

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strated that workers are divided into scouts and collectors, which duties however, may be interchanged at different periods. The collectors he divides into nectar collectors, pollen collectors and propolis collectors, and states that each group of collectors is, for the time being, faithful to its particular duty. Clements and Long (8) report that, in their own studies, "The analysis of pollen loads showed that 86 individuals carried mixed loads in comparison with 121 carrying pure pollen. With respect to species, none represented by five or more individuals was characterized by pure loads, the latter being found only on four bees belonging to *Halictus pulvenerus*, on three of *Monumetha albifrons*, and two of *Osmia melanotricha*. In *Apis* the relation of pure loads to mixed was 28 : 3, in *Andrena crataegi* 15 : 3, in *Bombus juxtus* 19 : 6, and in *Bifarius* 7 : 5. Variation in behavior within a genus is shown by the fact that for *B. occidentalis* the ratio was 9 : 17, *Andrena madronitens* 1 : 3, and *A. vicina* 2 : 3, while for *Halictus medionitens* it was 2 : 2. It is clear that bees in general are no more constant in the collection of pollen than in the gathering of nectar."

The question of whether bees remain constant to the same flower throughout its blooming period or whether the constancy applies only to individual bees is left unsettled by most of the earlier workers, or the two types of constancy are not clearly distinguished. In this connection some more recent observations may be quoted. Parker (17) observed hive bees at work on a mixed patch of boneset and aster; they confined themselves at one period to the boneset, at another to aster. Occasionally they alighted accidentally on the flowers which they were not working at the time, but immediately left them. The same behavior was noted in mixtures of *Spiraea* and *Lonicera*; of *Philadelphus* and *Physocarpus*; and of *Taraxacum* and *Melilotus*. However, it was also observed that bees occasionally visited both white and alsike clover impartially on the same trip. Philp and Vansell (19) state that the time of day influences availability of both nectar and pollen, so that activities shift during the day from one plant to the other. They have noted that filaree, in the orchards, is very attractive in the early morning, but most of the blossoms close by 10 o'clock on sunny days, and that some plants secrete nectar most abundantly during the night, the bees harvesting this in the morning and then shifting to some other plant. They declare that, in some seasons, the shift from prunes to mustard was most noticeable.

Miss Betts (5) has published results of observations on the constancy of pollen gathering bees, from microscopic examination of the loads of bees collected at the alighting board. She classifies mixed loads into two kinds; viz., "S" (segregated) and "M" (mixed) mixtures. In the first case the two kinds of pollen are distinct, this type of load resulting from the bee having collected part of its load from one plant and then switched to another. In the second the two kinds are mingled and can only be recognized as a mixture under the microscope. "S" mixture constituted 40% of the 233 loads examined, but it was considered that the preponderance of "M" mixtures was much greater. Mixtures of three or more kinds only constituted 6.5% of all



the mixtures examined and contained a larger proportion of garden plants than the average (47% as against 18% for two-species mixtures). She states that her specimens were collected, "with a view to securing as many mixtures as possible, so that the percentage—6.75%—of mixed to pure loads examined is plainly much higher than it is in nature."

The well-known researches of Von Frisch (11) and Von Frisch and Röscher (12) indicate that the scout bees communicate the stimulation for pollen or nectar collecting to their followers by means of the food odor and the excitation of the "round dance" in the case of nectar collectors, odor and the "tail-wagging dance" in the case of the pollen collectors. According to these workers the scouts do not "lead" the workers to the source of food, but the latter, having once obtained the scent from the scouts, must search for it themselves. In doing so they are aided by the odors from the scent glands of the bees of the same hive visiting that particular bloom. Workers returning to the hive excite another group of workers to visit that particular plant species, either as pollen or nectar gatherers, as the case may be. Thus, as long as the supply lasts, the number of bees visiting it will increase until the maximum supply is available. Thereafter there will take place a gradual diminution in number, as the bees find the supply diminishing.

In general it may be said that the evidence from the literature seems to support the conclusion that where abundance of pasturage of a single attractive species is available bees will work that particular species almost exclusively, though they may shift from one species to another at different times of the day, when certain species are more attractive at certain definite periods. On the other hand, where a great variety of bloom is available, without a predominance of any one species, there is a greater tendency to gather mixed loads, but the evidence is conflicting as the extent to which this occurs.

### Definition of Terms

From a perusal of the literature it is evident that several distinct phenomena have been included in the term "constancy" as applied to the flower-visiting habits of bees, resulting in considerable confusion. The following three types of constancy may be noted:—

*Type 1.* The insect confines its visits entirely to one flower species. *Type 2.* The insect confines its visits to a single species as long as it is available, but later changes to other flowers. *Type 3.* The insect confines its attention to one species of flower for each load, but may shift from one flower to the other for different loads.

The terms (1) monotrophic, (2) oligotrophic and (3) polytrophic in referring to the habit of the insect in confining its attention to (1) a single species, (2) a few related species, or (3) visiting indifferently many unrelated species, are often employed, but it should be understood that a species may be polytrophic and yet given individuals of that species may exhibit constancy of either the second or third type indicated above. Thus it is well known that

bees from the same hive may at any one time be found visiting many different species of plants. Nevertheless, this does not alter the fact that each individual bee may be confining its attention to a single species.

Constancy of the first type is well exhibited by *Halictoides novaeangliae* Robertson, which, as far as is known, confines its attention to the pickerel weed (*Pontederia cordata* L.). Constancy of the second type has been designated "polydrome constancy" by Clements and Long (8), while the term "monodrome constancy" has been proposed for the third type. Apparently some workers consider the hive bee to exemplify the second type and some the third.

### Studies in the Constancy of Hive Bees, Bumble Bees and Solitary Bees to Apple Bloom

#### (a) General

The mechanism of constancy in solitary bees, if it exists, can hardly be identical with that in hive bees, since the form of organization of the latter is entirely different and the division of labor among workers claimed for the hive bees cannot therefore obtain among *Halicti* and *Andrenae*. However, in these studies, we are concerned with the problem of the extent to which bees in working the apple bloom, visit only that species and to what extent they carry mixed loads, also whether there is any difference in this respect among the different genera concerned in apple pollination. Whether the degree of constancy that exists is merely a matter of convenience in the greater availability of a particular species of flower, whether it is due to superior attractiveness with respect to color, odor or form at a particular time, or whether it is due to an inherited tendency or to a definite physiological response, are matters beyond the scope of this investigation. For this reason the present study is based on analyses of the pollen carried by the species concerned. It may be pointed out that where pure loads only are found, this does not necessarily prove inherent constancy, but may mean merely that at the time of collection the particular plant species was preferred because of greater availability or attractiveness of the pollen or nectar.

Furthermore, these studies refer to pollen collection only and not to nectar. Corroborative evidence from observing the visits of bees to flowers might have been desirable, but this is difficult with respect to hive bees, and still more so with solitary bees, not only on account of their small size, but on account of the rapidity of their movement. Both *Halicti* and *Andrenae* appear to fly more readily from one tree to another than do the hive bees. Both hive bees and bumble bees usually work the blossoms on a limb more consistently and tend to remain fairly long in one tree, hence their movement can, to a certain extent, be observed, but no really reliable data regarding solitary bees can be obtained in this manner.

#### (b) Observation Points

It will be useful in considering the results obtained in the following study to have a short description of the various "stations" from which observations were carried out.

*Macdonald College.* At this point there were available numerous sources of pollen in the large perennial border and surrounding wild herbaceous plants, shrubs and trees. Observations on various species of plants were made until the College orchard, which covers 30 acres, came into bloom, after which collections were confined to the apple.

*Kentville, N.S.* As at the preceding station a very varied source of bloom was available, but, in addition, there was a very large orchard area, 70 acres on the station property. All collections made at this station and the ones that follow, except the last, were made from the apple bloom.

*Scott's Bay and North River, N.S.* At these stations the orchard area was very small and other sources of pollen somewhat scant and scattered.

*Long Island, N.S.* Here there was a total of 96 acres of mature orchard distributed over a total land area of 640 acres. Besides cultivated land and woods there was considerable land in rough pasture containing rhododendrons, blueberries, etc.

*Wolfville, N.S.* Collections were made at Wolfville in early August during a period when there was a lack of abundant pasturage of any one plant, but clumps and scattered plants of Canada thistle, wild radish, fire weed, dog bane and other plants were available.

*Blomidon, N.S.* A large orchard area was available for pasturage at this station, in addition to which ravines and other uncultivated land furnished a limited amount of other bloom.

Material collected at other undesignated points is simply labelled "Kings Co."

### (c) Method

The work was commenced at Macdonald College early in May when the early plants in the perennial borders were in bloom, but before apple bloom was available. Pollen was first obtained from all available bloom and mounted on slides for comparison with material to be later taken from the bees. This was kept up throughout the period of the study, samples of pollen from new plants being obtained and examined as fast as they came into blossom. In this way it was possible to determine most of the different kinds of pollen, at least as far as the genus. Previous to the blossoming of the apple, bees were collected from all available bloom, but after the apple bloom appeared, all bee collections were confined to it, and all available species were collected. Pollen was removed from the body hairs with a scalpel, the process being usually carried out under a dissecting microscope. Temporary or permanent mounts were then made for later study and photographing.

While collections of male bees were made and analyses of their pollen loads performed, the analysis of the data disregards entirely collections made from the males, owing to the inconstancy of the males of many species, in which a considerable degree of constancy is exhibited by the females.

It should be emphasized that the true constancy of the species studied is not necessarily clearly indicated by the analyses presented, because they do

not show the proportion of foreign pollen to host pollen. In many cases, however, the collections, especially those from apple bloom, show a large proportion of the individual loads to belong to a single plant species. Hence, the actual constancy of all species as far as single loads are concerned, may be actually considerably greater than indicated by percentage figures of pure and mixed loads.

It should be further explained that, in these studies, special attention was given to the pollen adhering to the body hairs, since it was considered that this would have the most significance in pollination and it could readily be washed from the body with alcohol. By following this method, large samples were not obtained, but mixtures, when they occurred, were readily recognizable. On the other hand, mixtures of pollen from the corbiculae of hive bees or bumble bees may be difficult to detect, especially if they are of the "mixed" type mentioned by Miss Betts. The "segregated" type can be readily detected if the pollen masses are of different color. The manner of securing our samples for analyses may possibly account for the fact that a higher percentage of mixtures was found than some other workers have reported. One would expect less error from contamination than would occur were the bees caught at the hive entrance. Possibly some of the pollen reported, such as that of *Phleum pratense*, may represent an accidental contamination, as we have never noted bees visiting this plant. Nevertheless, it is well known that, at certain times, bees will deliberately collect all sorts of material including flour or even sawdust and it may be that the pollen was actually gathered by the bees.

#### (d) Host Plants

In the accompanying tables only the genus of the host species and the pollen species is given in most cases. This is partly for the purpose of brevity in compiling the tables and partly because our main interest was merely to establish whether the insects concerned carried pure or mixed loads, which is sufficiently indicated by the foregoing practice. When a bee was taken on a certain host and the pollen taken from the body hairs was obviously of the same genus, the presumption would be that it was collected on that particular host, but when a pollen mixture was obtained it was not always possible to be sure of the exact species of the foreign pollen, owing to the close similarity between pollen of different members of the same genus. If the bee was taken from apple and bore, in addition to apple pollen, that of *Vaccinium* or *Trifolium*, it was not possible to distinguish between the species of the foregoing genera. Neither was it necessary in order to classify the sample as a "mixed" load. In the case of *Taraxacum officinale* our task was easy, as the pollen of this plant does not resemble that of any other blossoming at the same time. In other cases, as with liliaceous hosts, it is even difficult to separate genera.

Accordingly, the following list shows only the plant species from which bees were actually collected.

TABLE I

LIST OF HOST PLANTS ON WHICH COLLECTIONS WERE MADE

Scientific name	Common name	Place of collection
<i>Acer platanoides</i> L.	Norway maple	Macdonald College, P.Q.
<i>Alyssum saxatile</i> L.	Alyssum	Kentville, N.S.
<i>Amelanchier canadensis</i> L.	June berry	Macdonald College, P.Q.
<i>Brassica oleraceae</i> L.	Cabbage	Kings Co., N.S.
<i>Caragana arborescens</i> Lam.	Siberian pea tree	Macdonald College, P.Q.
<i>Cerastium tomentosum</i> L.	Snow-in-summer	Macdonald College, P.Q.
<i>Chrysanthemum leucanthemum</i> L.	Ox-eye daisy	North River; Blomidon.
<i>Cornus canadensis</i> L.	Bunchberry	Blomidon; North River.
<i>Cirsium arvense</i> (L.) Scip.	Canada thistle	Wolfville, N.S.
<i>Daucus carota</i> L.	Wild carrot	Blomidon; Scott's Bay.
<i>Fragaria virginiana</i> (Duchesne)	Wild strawberry	Kentville, N.S.
<i>Leontodon autumnalis</i> L.	Fall dandelion	Wolfville, N.S.
<i>Lonicera tartarica</i> L.	Tartarian honeysuckle	Macdonald College, P.Q.
<i>Muscari botryoides</i> L.	Grape hyacinth	Macdonald College, P.Q.
<i>Narcissus pseudo-narcissus</i> L.	Daffodil	Macdonald College, P.Q.
<i>Narcissus poeticus</i> L.	Poet's narcissus	Macdonald College, P.Q.
<i>Prunus avium</i> L.	Cultivated sweet cherry	Macdonald College, P.Q.
<i>Prunus nigra</i> Ait.	Wild or Canada plum	Macdonald College, P.Q.
<i>Pyrus malus</i> (Hill) S. F. Gray	Apple	Kentville; Long Island.
<i>Rosa</i> spp.	Wild rose	Wolfville, N.S.
<i>Rubus</i> spp.	Blackberry	Kings Co., N.S.
<i>Raphanus raphanistrum</i> L.	Wild radish	Wolfville, N.S.
<i>Rhododendron canadense</i> (L.) B.S.P.	Rhodora	Kentville; Long Island.
<i>Salix incana</i> Schank	Willow	Macdonald College, P.Q.
<i>Sambucus aurea</i> Cowell	Golden elder	Kentville, N.S.
<i>Scilla sibirica</i> Andr.	Siberian squill	Macdonald College, P.Q.
<i>Solidago canadensis</i> L.	Goldenrod	Wolfville, N.S.
<i>Spiraea vanhouttei</i> Zabel	Spiraea vanhouttei	Macdonald College, P.Q.
<i>Taraxacum officinale</i> Weber	Common dandelion	All stations.
<i>Trifolium repens</i> L.	White clover	North River; Blomidon; Scott's Bay.
<i>Tulipa gesneriana</i> L.	Tulip	Macdonald College, P.Q.
<i>Vaccinium canadense</i> Kalm.	Blueberry	Blomidon; Scott's Bay.
<i>V. pennsylvanicum</i> Lam.	Blueberry	North River; Macdonald College, P.Q.

In Table II are listed the various species collected and the result of analysis of their pollen loads from all the stations. In cases in which a bee was taken on a certain plant, but bore pollen from another, we have counted this a "mixed visit," on the assumption that it had not yet had time to secure pollen from the plant on which it was found, in sufficient quantity to appear in the analyses.

TABLE II

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species	Date	No. examined	Host	Pollen species
<i>Andrena crataegi</i> Robt.—Macdonald College				<i>Andrena bradleyi</i> Vier—Kentville			
May 16	2	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Caragana</i>	May 30	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Rhododendron</i> (*)
May 16	2	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i>	<i>Andrena milwaukieensis</i> —Kentville			
May 16	4	<i>Prunus</i>	<i>Prunus</i>	June 4	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 16	2	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Tulipa</i>	<i>Andrena obscura</i> Robt.—Macdonald College			
May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i>	May 9	2	<i>Salix</i>	<i>Salix</i>
May 16	2	<i>Prunus</i>	<i>Prunus</i> ; <i>Spiraea</i> ; <i>Taraxacum</i>	May 12	1	<i>Salix</i>	<i>Tulipa</i>
May 19	1	<i>Spiraea</i>	<i>Taraxacum</i>	May 19	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i> ; <i>Tulipa</i>	June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Tulipa</i>	June 9	1	<i>Pyrus malus</i>	<i>Trifolium</i>
May 19	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ; <i>Tulipa</i> ; <i>Salix</i>	<i>Andrena rugosa</i> Robt.—Scott's Bay			
May 19	2	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ; <i>Tulipa</i>	June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 19	2	<i>Narcissus</i>	<i>Tulipa</i> ; <i>Salix</i>	June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
May 19	3	<i>Narcissus</i>	<i>Tulipa</i>	<i>Andrena thaspis</i> Graen.—North River			
May 19	1	<i>Narcissus</i>	<i>Prunus</i> ; <i>Salix</i>	June 9	7	<i>Pyrus malus</i>	<i>Vaccinium</i>
May 19	1	<i>Amelanchier</i>	<i>Taraxacum</i> ; <i>Amelanchier</i> ; <i>Spiraea</i>	June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
May 19	3	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Tulipa</i> ; <i>N. pseudo-narcissus</i>	June 9	2	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Vaccinium</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Taraxacum</i> ; <i>Tulipa</i> ; <i>N. pseudo-narcissus</i>	June 9	2	<i>Pyrus malus</i>	<i>Vaccinium</i> ; <i>Trifolium</i>
May 19	2	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Tulipa</i> ; <i>Taraxacum</i>	June 9	3	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Chrysanthemum</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i>	June 9	1	<i>Pyrus malus</i>	<i>Chrysanthemum</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Caragana</i> ; <i>Prunus</i>	June 9	4	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i> ; <i>N. pseudo-narcissus</i>	<i>Andrena wilkella</i> Kirby—North River			
<i>Andrena crataegi</i> Robt.—North River				June 9	1	<i>Pyrus malus</i>	<i>Pinus</i>
June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i> ; <i>Trifolium</i>	<i>Andrena wilkella</i> Kirby—Long Island			
<i>Andrena crataegi</i> Robt.—Blomidon				June 9	5	<i>Pyrus malus</i>	<i>Pyrus malus</i>
June 13	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Andrena wilkella</i> Kirby—Blomidon			
<i>Andrena annae</i> Ckll.—Macdonald College				June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 19	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	June 12	3	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Andrena carlini</i> Ckll.—Kentville				June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Daucus carota</i>
May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Andrena wilkella</i> Kirby—Scott's Bay			
May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Tragacantha</i>	June 12	30	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 30	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 12	12	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
<i>Andrena carlini</i> Ckll.—Blomidon				June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>
June 12	3	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Andrena wilkella</i> Kirby—Wolfville			
June 12	2	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>	July 30	1	<i>Cirsium</i>	<i>Cirsium</i>
June 13	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	Aug. 1	3	<i>Solidago</i>	<i>Solidago</i>
<i>Andrena sp.</i> —Kentville				<i>Andrena sp.</i> —Macdonald College			
May 25	7	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 9	1	<i>Salix</i>	<i>Salix</i> ; <i>N. pseudo-narcissus</i>
May 25	1	<i>Pyrus malus</i>	<i>Sambucus</i>	May 12	2	<i>Salix</i>	<i>Salix</i> ; <i>Tulipa</i>
May 25	1	<i>Pyrus malus</i>	<i>Taraxacum</i>	May 12	1	<i>Salix</i>	<i>Tulipa</i>
				May 16	1	<i>Acer</i>	<i>Tulipa</i>
				May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i>
				May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Phlox subulata</i>
				May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i>
				May 19	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
				May 19	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Lonicera</i>
				May 19	1	<i>Spiraea</i>	<i>Tulipa</i>

(\*) *Rhododendron* predominating.

TABLE II—Continued

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species	Date	No. examined	Host	Pollen species
<i>Andrena</i> sp.—Kentville				<i>Apis mellifica</i> L.—Macdonald College—Concluded			
May 30	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	May 12	1	<i>Salix</i>	<i>N. pseudo-narcissus</i>
<i>Andrena</i> sp.—Macdonald College				May 16	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>N. pseudo-narcissus</i>
June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i>	May 16	2	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Tulipa</i>
June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Pyrus malus</i>	May 16	2	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Tulipa</i> ; <i>N. pseudo-narcissus</i>
June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Caragana</i>	May 16	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Salix</i>
<i>Andrena</i> sp.—North River				May 16	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Tulipa</i> ; <i>Salix</i>
June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Vaccinium</i> ; <i>Trifolium</i> <i>Chrysanthemum</i>	May 16	2	<i>Taraxacum</i>	<i>N. pseudo-narcissus</i> ; <i>Tulipa</i> ; <i>Taraxacum</i> ; <i>Salix</i>
June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Vaccinium</i>	May 16	1	<i>Taraxacum</i>	<i>N. pseudo-narcissus</i> ; <i>Crocus</i> ; <i>Taraxacum</i> ; <i>Tulipa</i> ; <i>Salix</i>
June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i>	May 16	1	<i>Taraxacum</i>	<i>Salix</i> ; <i>Tulipa</i>
June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 16	1	<i>Taraxacum</i>	<i>Salix</i> ; <i>N. pseudo-narcissus</i> ; <i>Tulipa</i>
<i>Andrena</i> sp.—Scott's Bay				May 16	1	<i>Taraxacum</i>	<i>Tulipa</i> (†)
June 16	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Tulipa</i> ; <i>Lonicera</i>
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	May 16	1	<i>Prunus</i>	<i>Tulipa</i> ; <i>Prunus</i>
<i>Andrena vicina</i> Smith—Macdonald College				May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i> ; <i>Phlox subulata</i>
May 9	1	<i>Scilla</i>	<i>Scilla</i> ; <i>Taraxacum</i>	May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i>
May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Caragana</i>	May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i>
May 16	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Vaccinium</i>	May 16	1	<i>Acer</i>	<i>Tulipa</i>
May 19	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 16	1	<i>Acer</i>	<i>Tulipa</i>
May 19	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Prunus</i>	May 16	1	<i>Acer</i>	<i>Tulipa</i>
May 19	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i> ; <i>Tulipa</i>	May 19	2	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> (‡)
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i> ; <i>Tulipa</i>	May 19	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i> ; <i>Lonicera</i>
May 31	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i>	May 19	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Caragana</i>
<i>Andrena vicina</i> Smith—North River				May 19	1	<i>Narcissus</i>	<i>Tulipa</i>
June 9	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 19	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>	May 19	2	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
<i>Apis mellifica</i> L.—Macdonald College				May 19	3	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i>
May 9	1	<i>Salix</i>	<i>Salix</i> ; <i>Taraxacum</i> (*)	May 29	7	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 9	3	<i>Salix</i>	<i>Salix</i>	May 29	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Tulipa</i>
May 9	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i>	May 31	1	<i>Prunus</i>	<i>Prunus</i> ; <i>Taraxacum</i>
May 12	1	<i>Salix</i>	<i>Salix</i> ; <i>N. poeticus</i>	May 31	3	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 12	2	<i>Salix</i>	<i>Taraxacum</i> ; <i>N. pseudo-narcissus</i> ; <i>Salix</i>	May 31	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Caragana</i>
May 12	7	<i>Salix</i>	<i>Salix</i> ; <i>N. pseudo-narcissus</i>	May 31	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
May 12	8	<i>Salix</i>	<i>Salix</i> ; <i>Tulipa</i>	June 1	4	<i>Taraxacum</i>	<i>Taraxacum</i>
May 12	7	<i>Salix</i>	<i>Salix</i> ; <i>N. pseudo-narcissus</i> ; <i>Tulipa</i>	June 1	7	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Pyrus malus</i>
May 12	4	<i>Salix</i>	<i>Tulipa</i>	June 2	8	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 12	1	<i>Salix</i>	<i>N. pseudo-narcissus</i> ; <i>Tulipa</i>	June 2	3	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
May 12	2	<i>Salix</i>	<i>Salix</i> ; <i>Tulipa</i> ; <i>Taraxacum</i>				

(\*) Pollen scarce; bees collected on (*Salix*) pistillate tree. Staminate tree in full bloom.

(†) Pollen packed.

(‡) Collected before full apple bloom.

TABLE II—Continued

## LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species	Date	No. examined	Host	Pollen species
<i>Apis mellifica</i> L.—Kentville				<i>Bremus fervidus</i> Fob.—Macdonald College—Concluded			
May 25	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 25	2	<i>Lonicera</i>	<i>Lonicera</i> ; <i>Taraxacum</i> ;
May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;				<i>Caragana</i>
			<i>Taraxacum</i>	(*) May 25	1	<i>Lonicera</i>	<i>Lonicera</i>
May 30	43	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Bremus fervidus</i> Fob.—Blomidon			
May 30	7	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;	June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Phleum</i> ;
			<i>Taraxacum</i>				<i>Daucus carota</i>
<i>Apis mellifica</i> L.—Long Island				June 13	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Daucus carota</i>
June 4	25	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 16	3	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Phleum</i>
June 9	21	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 16	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Apis mellifica</i> L.—North River				<i>Bremus fervidus</i> Fob.—Scott's Bay			
June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;	June 16	2	<i>Pyrus malus</i>	<i>Phleum</i>
			<i>Vaccinium</i> ;	<i>Bremus ternarius</i> Say.—Macdonald College			
			<i>Trifolium</i>	May 12	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ;
June 11	2	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;				<i>Tulipa</i>
			<i>Vaccinium</i>	June 2	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Bremus ternarius</i> Say.—Kentville			
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;	May 25	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
			<i>Vaccinium</i> ; <i>Cornus canadensis</i>	May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;				<i>Rhododendron</i>
			<i>Vaccinium</i> ; <i>Cornus canadensis</i> ; <i>Phleum</i>	June 4	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
June 12	5	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;				<i>Taraxacum</i>
			<i>Vaccinium</i>	<i>Bremus ternarius</i> Say.—Long Island			
June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;	May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
			<i>Vaccinium</i> ;	May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
			<i>Chrysanthemum</i>				<i>Rhododendron</i>
June 12	2	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;	June 4	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
			<i>Chrysanthemum</i>				<i>Rhododendron</i>
June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Bremus ternarius</i> Say.—Blomidon			
<i>Apis mellifica</i> L.—Blomidon				June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
June 13	5	<i>Pyrus malus</i>	<i>Pyrus malus</i>				<i>Chrysanthemum</i>
<i>Apis mellifica</i> L.—Wolfville				June 13	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ;
July 30	9	<i>Cirsium</i>	<i>Cirsium</i>				<i>Daucus carota</i>
Aug. 1	3	<i>Solidago</i>	<i>Solidago</i>	<i>Bremus ternarius</i> Say.—Scott's Bay			
Aug. 1	1	<i>Rosa</i>	<i>Rosa</i> ; <i>Leontodon</i>	June 16	8	<i>Pyrus malus</i>	<i>Pyrus malus</i>
Aug. 10	22	<i>Raphanus</i>	<i>Raphanus</i>	June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Pinus</i>
Aug. 10	1	<i>Raphanus</i>	<i>Raphanus</i> ; <i>Leontodon</i>	June 16	1	<i>Pyrus malus</i>	<i>Vaccinium</i>
Aug. 10	3	<i>Solidago</i>	<i>Solidago</i>	<i>Bremus ternarius</i> Say.—Wolfville			
<i>Apis mellifica</i> L.—Scott's Bay				July 30	1	<i>Cirsium</i>	<i>Cirsium</i>
June 16	10	<i>Pyrus malus</i>	<i>Pyrus malus</i>	Aug. 1	1	<i>Solidago</i>	<i>Cirsium</i>
<i>Bremus borealis</i> Kirby—Scott's Bay				<i>Bremus terricola</i> Kirby—Macdonald College			
June 16	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 9	1	<i>Salix</i>	<i>Salix</i>
<i>Bremus fervidus</i> Fob.—Macdonald College				May 25	1	<i>Lonicera</i>	<i>Lonicera</i>
May 12	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ;	<i>Bremus terricola</i> Kirby—Blomidon			
			<i>Tulipa</i>	June 13	1	<i>Pyrus malus</i>	<i>Pinus</i> ; <i>Phleum</i>
May 12	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ;	<i>Bremus terricola</i> Kirby—Scott's Bay			
			<i>Tulipa</i> ; <i>Salix</i> ;	June 13	3	<i>Pyrus malus</i>	<i>Pyrus malus</i>
			<i>Taraxacum</i>	<i>Bremus vagans</i> Smith—Macdonald College			
May 12	1	<i>Narcissus</i>	<i>Taraxacum</i> ; <i>Tulipa</i> ;	May 9	1	<i>Salix</i>	<i>Salix</i> ; <i>N. pseudo-narcissus</i>
			<i>N. pseudo-narcissus</i>	May 9	1	<i>Salix</i>	<i>Taraxacum</i>
May 9	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i>	May 9	1	<i>Salix</i>	<i>Salix</i>
May 9	1	<i>Muscari</i>	<i>Muscari</i>	May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i>
May 19	1	<i>Amelanchier</i>	<i>Amelanchier</i> ; <i>Tulipa</i>	May 21	1	<i>Caragana</i>	<i>Caragana</i> ; <i>Tulipa</i>

(\*) *Pyrus* predominating.(†) Three with *Pyrus* predominating.



TABLE II—Continued

## LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species	Date	No. examined	Host	Pollen species
<i>Bremus vagans</i> Smith—North River				<i>Halictus foxii</i> Robt.—Kentville			
July 9	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Bremus vagans</i> Smith—Scott's Bay				<i>Halictus foxii</i> Robt.—Macdonald College			
May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i>
June 16	18	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Halictus foxii</i> Robt.—North River			
June 16	1	<i>Pyrus malus</i>	<i>Phleum</i> ; <i>Pinus</i>	June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i>
June 16	1	<i>Pyrus malus</i>	<i>Pinus</i>	June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Vaccinium</i>	June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>
June 16	1	<i>Pyrus malus</i>	<i>Vaccinium</i>	<i>Halictus foxii</i> Robt.—Blomidon			
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Vaccinium</i> ; <i>Phleum</i>	June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Phleum</i>
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Daucus carota</i>	<i>Halictus foxii</i> Robt.—Scott's Bay			
<i>Bremus</i> sp.—Macdonald College				June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 19	1	<i>Spiraea</i>	<i>Spiraea</i> ; <i>Prunus</i> ; <i>Taraxacum</i>	<i>Halictus foxii</i> Robt.—Wolfville			
May 20	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	June, 1932	1	<i>Rubus</i>	<i>Rubus</i>
May 20	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June, 1932	1	<i>Brassica</i>	<i>Brassica</i>
May 29	1	<i>Prunus</i>	<i>Prunus</i>	June, 1932	1	<i>Rubus</i>	<i>Rubus</i> ; unknown sp. (‡)
<i>Bremus</i> sp.—Blomidon				<i>Halictus lerouxii</i> LeP.—Macdonald College			
June 12	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Phleum</i> ; <i>Vaccinium</i> ; <i>Chrysanthemum</i>	May 12	1	<i>Salix</i>	<i>Tulipa</i>
<i>Halictus arcuatus</i> Robt.—Kentville				<i>Halictus lerouxii</i> LeP.—Kentville			
May 25	5	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 25	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	<i>Halictus lerouxii</i> LeP.—Long Island			
<i>Halictus craterus</i> Lov.—North River				June 4	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
June 9	1	<i>Pyrus malus</i>	<i>Chrysanthemum</i> ; <i>Trifolium</i>	<i>Halictus lerouxii</i> LeP.—Blomidon			
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 13	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Halictus craterus</i> Lov.—Wolfville				<i>Halictus lerouxii</i> LeP.—Scott's Bay			
July 30	31	<i>Cirsium</i>	<i>Cirsium</i>	June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
July 30	1	<i>Cirsium</i>	<i>Cirsium</i> ; <i>Lychnus</i>	<i>Halictus lerouxii</i> LeP.—Wolfville			
<i>Halictus craterus</i> Lov.—Wolfville				Aug. 1	1	<i>Solidago</i>	<i>Solidago</i>
Aug. 6	107	<i>Leonodon</i>	<i>Leonodon</i>	<i>Halictus macoupinensis</i> —Kings Co.			
Aug. 6	1	<i>Rosa</i>	<i>Rosa</i> ; <i>Leonodon</i> ; unknown sp. (*)	June, 1932	2	<i>Rubus</i>	<i>Rubus</i>
Aug. 6	1	<i>Rosa</i>	<i>Leonodon</i> ; unknown sp. (*)	June, 1932	3	<i>Brassica</i>	<i>Brassica</i>
Aug. 6	1	<i>Rosa</i>	<i>Leonodon</i> ; <i>Rosa</i> (*)	June, 1932	1	<i>Rubus</i>	<i>Rubus</i> ; unknown sp.
Aug. 6	3	<i>Rosa</i>	<i>Rosa</i>	<i>Halictus pectoralis</i> —Kings Co.			
<i>Halictus cressonii</i> Robt.—Blomidon				Aug. 1	1	<i>Solidago</i>	<i>Solidago</i>
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Phleum</i>	<i>Halictus pilosus</i> Smith—Kentville			
<i>Halictus cressonii</i> Robt.—Scott's Bay				May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>
June 16	4	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Halictus pilosus</i> Smith—Kings Co.			
<i>Halictus cressonii</i> Robt.—Kings Co.				June, 1932	1	<i>Brassica</i>	<i>Brassica</i>
June, 1932	4	<i>Brassica</i>	<i>Brassica</i>	<i>Halictus provancheri</i> D. T.—Macdonald College			
June, 1932	1	<i>Rhododendron</i>	<i>Rhododendron</i> ; <i>Pyrus malus</i> (†)	May 9	1	<i>Salix</i>	<i>Salix</i>
<i>Halictus coriaceus</i> Smith—Scott's Bay				May 9	1	<i>Cerastium</i>	<i>Cerastium</i>
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 9	4	<i>Scilla</i>	<i>Scilla</i>
				<i>Halictus provancheri</i> D. T.—Scott's Bay			
				June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>
				<i>Halictus provancheri</i> D. T.—Wolfville			
				July 30	1	<i>Cirsium</i>	<i>Cirsium</i>
				<i>Halictus provancheri</i> D. T.—Kings Co.			
				June, 1932	1	<i>Rubus</i>	<i>Rubus</i> ; unknown sp.
				June, 1932	1	<i>Brassica</i>	<i>Brassica</i> ; unknown sp.

(\*) *Leonodon* predominating.(†) *Pyrus* predominating.(‡) *Rubus* predominating.

TABLE II—*Concluded*

## LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species	Date	No. examined	Host	Pollen species
<i>Halictus smilacinae</i> Robt.—Kentville				<i>Halictus</i> sp.—Macdonald College— <i>Concluded</i>			
May 25	14	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 12	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i>
May 25	6	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Taraxacum</i>	May 12	1	<i>Narcissus</i>	<i>Tulipa</i>
			(*)	May 12	1	<i>Narcissus</i>	<i>N. pseudo-narcissus</i> ; <i>Tulipa</i> ; <i>Salix</i>
<i>Halictus smilacinae</i> Robt.—Long Island				May 19	1	<i>Amelanchier</i>	<i>Spiraea</i> ; <i>Amelanchier</i>
June 4	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i>
<i>Halictus smilacinae</i> Robt.—Scott's Bay				June 1	1	<i>Taraxacum</i>	<i>Taraxacum</i> ; <i>Caragana</i>
June 9	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>	June 16	2	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Halictus smilacinae</i> Robt.—Blomidon				<i>Halictus</i> sp.—Kentville			
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i> ; <i>Vaccinium</i>	May 25	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
June 11	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	<i>Ceratina</i> sp.—Macdonald College			
June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>	May 9	1	<i>Amelanchier</i>	<i>Amelanchier</i>
<i>Halictus smilacinae</i> Robt.—Wolfville				<i>Colletes</i> sp.—Wolfville			
July 30	1	<i>Cirsium</i>	<i>Cirsium</i>	Aug. 1	4	<i>Solidago</i>	<i>Solidago</i>
Aug. 1	6	<i>Solidago</i>	<i>Solidago</i>	Aug. 1	1	<i>Solidago</i>	<i>Solidago</i> ; <i>Rosa</i>
Aug. 1	1	<i>Solidago</i>	<i>Solidago</i> ; <i>Rosa</i>	<i>Megachile</i> sp.—Scott's Bay			
Aug. 1	2	<i>Solidago</i>	<i>Solidago</i> ; unknown sp.	June 16	1	<i>Pyrus malus</i>	<i>Pyrus malus</i>
<i>Halictus smilacinae</i> Robt.—Kings Co.				<i>Megachile</i> sp.—Wolfville			
June, 1932	1	<i>Brassica</i>	<i>Brassica</i> ; 2 unknown sp.	July 30	1	<i>Cirsium</i>	<i>Cirsium</i> ; <i>Raphanus</i>
June, 1932	3	<i>Brassica</i>	<i>Brassica</i>	July 30	1	<i>Cirsium</i>	<i>Cirsium</i>
June, 1932	3	<i>Brassica</i>	<i>Brassica</i> ; 1 unknown sp.	July 30	1	<i>Solidago</i>	<i>Solidago</i>
June, 1932	2	<i>Brassica</i>	Unknown sp.	<i>Mellisoides</i> sp.—Wolfville			
June, 1932	1	<i>Taraxacum</i>	<i>Taraxacum</i>	Aug. 1	2	<i>Solidago</i>	<i>Solidago</i>
June, 1932	1	<i>Prunus</i>	<i>Prunus</i>	Aug. 1	1	<i>Solidago</i>	<i>Solidago</i> ; <i>Rosa</i>
<i>Halictus</i> sp.—Macdonald College				Aug. 6	1	<i>Leontodon</i>	<i>Leontodon</i>
May 9	2	<i>Scilla</i>	<i>Scilla</i>	<i>Osmia</i> sp.—Macdonald College			
May 9	1	<i>Muscari</i>	<i>Tulipa</i>	June 9	1	<i>Pyrus malus</i>	<i>Trifolium</i>
May 9	1	<i>Salix</i>	<i>Salix</i>	June 9	1	<i>Pyrus malus</i>	<i>Pyrus malus</i> ; <i>Trifolium</i>
May 9	1	<i>Cerastium</i>	<i>Cerastium</i>	June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i> ; <i>Pyrus malus</i> ; <i>Chrysanthemum</i>
May 9	2	<i>Taraxacum</i>	<i>Taraxacum</i>	<i>Spechodes</i> —Macdonald College			
				June 9	1	<i>Pyrus malus</i>	<i>Vaccinium</i>

(\*) *Pyrus* predominating in all but one case.

Based on the foregoing, Table III has been prepared in which have been tabulated (1) the totals of pure and mixed loads collected from all species of plants by representatives of the four genera, *Andrena*, *Apis*, *Bremus*, and *Halictus*, (2) the summarized results from all stations applied to apple visitors only, (3) the results from mixed collections at Macdonald College previous to bloom, (4) the separate results for apple visitors only from Macdonald College, Kentville, Long Island, Scott's Bay and Blomidon, and (5) the results from mixed collections at Wolfville made during the early part of August.

While still larger numbers of individual collections would have been preferable, particularly during apple bloom, the data, thus made available, do furnish useful information regarding the comparative constancy of the species taken under varied conditions.

TABLE III

## FLOWER CONSTANCY OF HIVE AND WILD BEES

Genus	Observation station	Host plant	Pure loads		Mixed visits				Total
			No	%	2 spp	3 spp	4 spp	5 spp	
<i>Andrena</i>	All stations	All species	82	45 50	70	26	2	—	98
<i>Apis</i>	All stations	All species	163	61 97	73	22	4	1	100
<i>Bremus</i>	All stations	All species	50	58 80	25	8	2	—	35
<i>Halictus</i>	All stations	All species	207	83 90	34	6	—	—	40
<i>Andrena</i>	All stations	<i>Pyrus malus</i>	71	57 20	47	5	1	—	53
<i>Apis</i>	All stations	<i>Pyrus malus</i>	118	80 30	25	3	1	—	29
<i>Bremus</i>	All stations	<i>Pyrus malus</i>	41	65 00	17	4	1	—	22
<i>Halictus</i>	All stations	<i>Pyrus malus</i>	41	71 90	14	2	—	—	16
<i>Andrena</i>	Macdonald College	Mixed species	15	21 70	28	24	2	—	54
<i>Apis</i>	Macdonald College	Mixed species	28	26 60	55	18	3	1	77
<i>Bremus</i>	Macdonald College	Mixed species	19	59 40	88	4	—	1	13
<i>Halictus</i>	Macdonald College	Mixed species	18	81 80	4	—	—	—	4
<i>Andrena</i>	Macdonald College	<i>Pyrus malus</i>	6	60 0	3	1	—	—	4
<i>Apis</i>	Macdonald College	<i>Pyrus malus</i>	20	71 4	8	—	—	—	8
<i>Bremus</i>	Macdonald College	<i>Pyrus malus</i>	2	66 6	1	—	—	—	1
<i>Halictus</i>	Macdonald College	<i>Pyrus malus</i>	—	—	—	—	—	—	—
<i>Andrena</i>	Kentville	<i>Pyrus malus</i>	11	68 75	5	—	—	—	5
<i>Apis</i>	Kentville	<i>Pyrus malus</i>	45	84 9	8	—	—	—	8
<i>Bremus</i>	Kentville	<i>Pyrus malus</i>	3	60 0	2	—	—	—	2
<i>Halictus</i>	Kentville	<i>Pyrus malus</i>	24	77 4	7	—	—	—	7
<i>Andrena</i>	Long Island	<i>Pyrus malus</i>	5	100 0	—	—	—	—	—
<i>Apis</i>	Long Island	<i>Pyrus malus</i>	46	100 0	—	—	—	—	—
<i>Bremus</i>	Long Island	<i>Pyrus malus</i>	1	33 3	2	—	—	—	2
<i>Halictus</i>	Long Island	<i>Pyrus malus</i>	3	100 0	—	—	—	—	—
<i>Andrena</i>	North River	<i>Pyrus malus</i>	6	21 4	18	3	1	—	22
<i>Apis</i>	North River	<i>Pyrus malus</i>	2	14 3	9	2	1	—	12
<i>Bremus</i>	North River	<i>Pyrus malus</i>	2	100 0	—	—	—	—	—
<i>Halictus</i>	North River	<i>Pyrus malus</i>	1	25 0	3	1	—	—	4
<i>Andrena</i>	Scott's Bay	<i>Pyrus malus</i>	33	67 4	16	—	—	—	16
<i>Apis</i>	Scott's Bay	<i>Pyrus malus</i>	10	100 0	—	—	—	—	—
<i>Bremus</i>	Scott's Bay	<i>Pyrus malus</i>	32	76 3	9	1	—	—	10
<i>Halictus</i>	Scott's Bay	<i>Pyrus malus</i>	9	90 0	1	—	—	—	1
<i>Andrena</i>	Blomidon	<i>Pyrus malus</i>	11	78 6	3	—	—	—	3
<i>Apis</i>	Blomidon	<i>Pyrus malus</i>	5	100 0	—	—	—	—	—
<i>Bremus</i>	Blomidon	<i>Pyrus malus</i>	2	16 7	6	3	1	—	10
<i>Halictus</i>	Blomidon	<i>Pyrus malus</i>	3	37 5	2	2	1	—	5
<i>Andrena</i>	Wolfville	All species	4	100 0	—	—	—	—	—
<i>Apis</i>	Wolfville	All species	37	95 0	2	—	—	—	2
<i>Bremus</i>	Wolfville	All species	2	100 0	—	—	—	—	—
<i>Halictus</i>	Wolfville	All species	150	88 8	16	3	—	—	19

## Comments on Results

It would appear from the results from all stations that, considering the high degree of error inherent in calculations based on such comparatively small numbers, none of the bee species show as high a degree of constancy as some workers have claimed, when the records from all flowers visited are

studied. The difference between the genera when considered in this way, is not particularly significant. Considering the data from all stations and from all host species *Halictus* shows the highest degree of constancy, followed by *Apis*, *Bremus* and *Andrena*. Considering only the data secured from material collected during apple bloom, *Apis* appears relatively more constant and *Halictus* somewhat less so, while following them in order we have *Bremus* and *Andrena*. In all cases the different species show 50% or more of the individuals collected carrying pure loads. Considering only results from the great variety of bloom available at Macdonald College, *Halictus* shows greatest constancy and *Andrena* least, with *Bremus* and *Apis* in order following an intermediate position. At Wolfville, also from mixed bloom and neglecting *Bremus* and *Andrena* because of the insignificant number collected, we have *Apis* again taking first place, followed by *Halictus*, both showing a high degree of constancy.

The figures from the various individual stations are too small, especially in the case of wild bees, to draw any sweeping conclusions, but they indicate, with one unimportant exception, that, when abundant apple bloom is available, all species show a degree of constancy exceeding 50% and sometimes reaching 100%. The figures do not permit us to conclude with any degree of certainty that any of the species studied show any decided advantage over the others in regard to constancy, except that *Apis* and *Halictus* appear, on the whole to have an advantage. When abundant bloom of many different species was available, as at Macdonald College, without a predominance of any one plant species, the constancy exhibited was generally less for all genera. Though records at Wolfville were taken from mixed bloom the conditions were somewhat different to those at Macdonald College, since the number of plant species available was less. This is reflected in results obtained, which show a much greater degree of constancy than in the spring counts. At this station we have significant numbers only for *Halictus* and *Apis*, both showing a high degree of constancy, with the margin in favor of the latter.

It would thus appear that the degree of constancy exhibited by the insects concerned varies considerably with the situation, availability and attractiveness being important factors. Where large exposures of bloom of an attractive species are available all forms studied appear to prefer to follow a single host. The degree of constancy became less, the greater the multiplicity of hosts, with an insufficiency of any particular one to furnish adequate pasturage. It would also appear that, by selecting particular periods of the year and a particular host distribution, one could obtain almost any result desired, with respect to the constancy of any species.

### Summary

1. This investigation was undertaken primarily to determine the relative pollen constancy of hive bees and wild bees to apple bloom and, secondarily, to various spring and summer blooming plants.

2. The observations were made at Macdonald College, Que., and at various stations in Kings Co., Nova Scotia.

3. Pollen for analysis was obtained both from the body hairs and from the corbiculae of the bees.

4. Previous to apple bloom collections of bees were made on a wide range of hosts. During the apple blossoming period collections were confined to that species. Late summer collections were made mainly from the Compositae.

5. Constancy in all species bore a direct relationship to the relative abundance of different types of bloom.

6. Generic differences in constancy were not particularly significant. During the period of apple bloom *Apis* appeared to be the most constant, but through the entire season the *Halicti* exhibited the highest average constancy.

7. In the pollen analyses, the species present were recorded without estimation of the relative amount of each kind represented. Had this been done the degree of constancy for all species would have appeared higher.

### Acknowledgments

Miss Janie Matheson carried out the details of the work of pollen analyses at Macdonald College and a part of those in Nova Scotia. Mr. Robert Ward and Mr. John Leefe carried out a large proportion of the analyses made in Nova Scotia. Professor J. G. Coulson, Macdonald College, provided laboratory space and gave freely of his time in connection with the photographing of the pollen samples taken at Macdonald College. Identification of the bees used in our studies was made by Mr. C. E. Atwood. In this he was greatly assisted by material of *Halicti* determined by Miss Grace Sandhouse, Bureau of Entomology, Washington, and of *Andrenae* by Dr. T. D. A. Cockrell, Boulder, Colorado.

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## VARIATION IN *CLOSTRIDIUM WELCHII*<sup>1</sup>

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### Abstract

In this paper it is shown that by plating cultures of *Cl. welchii*, on a suitable hormone-blood agar, two distinct colony types, regarded as S and R, and frequently intermediate types, may be isolated. Once isolated and stabilized by continued cultivation and selection at least the two more extreme types show true breeding properties. Serial cultivation in various fluid media, particularly Robertson's chopped meat, has resulted in both S to R and R to S variation. This has been observed in strains purified by colony selection and by single cell isolation.

The S type has proved to be highly virulent for pigeons, the R type much less virulent but there is some evidence that the apparent killing action of R types may result from *in vivo* dissociation of the R to S. Both S and R types are shown to yield hemotoxin which is neutralized by antitoxin but under similar conditions S types produce 10 to 20 times more toxin than R types.

### Introduction

In recent work on the *in vitro* and *in vivo* action of *Cl. welchii* (Reed, Orr and Burleigh (7) and Orr, Campbell and Reed (5)) much difficulty was experienced owing to variation in the pathogenicity of the same strain of organisms at different times and in the potency of different lots of toxin apparently prepared in the same manner. Two possible explanations suggest themselves: either, variation of the organisms or difference in toxin production as a result of possible differences in successive lots of the culture media. The influence of the medium will be discussed in a subsequent paper.

In a preliminary paper (6), it was shown that *Cl. welchii* displays variation or dissociation to a conspicuous degree and that by a process of selection at least two variant types may be isolated from pure cultures, types which differ in colony structure, pathogenicity and toxin production. In the present paper more detailed evidence of this variation is presented. The only instances of this sort of variation among the anaërobic bacteria which appear to have been reported are short notes by Sordelli and Ferrari (9) on *Cl. septicæ*, and Spray (10) on several species. Personal communications from Professors Hadley of Michigan and Maitland of Manchester indicate that they have made similar observations.

### Experimental Methods

The several cultures of *Cl. welchii* used in this investigation had been carried in stock for a number of generations, in a chopped-beef infusion broth medium. Dr. I. C. Hall of the University of Colorado very kindly sent to us two further strains designated C<sub>4</sub> and C<sub>5</sub>.

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In the study of variation based initially on colony structure, cultures were made on plates of a hormone agar prepared essentially as described by Huntoon (1) enriched with 0.1% of glucose and 5% of defibrinated rabbit blood. The plates were incubated at 37.5° C. in Fildes jars; phosphorus jars, as modified by Varney (11); or in pyrogallol jars. The former, especially when calcium chloride was used in the bottom of the jar in order to ensure that the medium had a relatively dry surface, proved most convenient although equally good results were obtained by the three methods. An incubation period of 48 hr. was found to give the most distinctive colony forms. The inoculum was always very light in order to ensure the development of discrete colonies; best results were obtained with plates showing not more than 100 to 200 colonies. On this medium and under these conditions, growth of *Cl. welchii* was much more luxuriant and colonies were much larger than those which have generally been described. When plates of this medium and blood medium made with ordinary beef-extract agar were inoculated from the same emulsion, the former yielded colonies several times larger than the latter even when the two were incubated in the same anaërobic jar. Moreover, where variation occurred the differences in colony form were very much more conspicuous on the hormone than on the ordinary blood agar.

### Two Colony Forms

By the procedure outlined it was possible to isolate at least two types of colonies from all of the cultures studied. One, considered the S type, resembles the colony form usually described as characteristic of *Cl. welchii* (4, 8, 12, 14). This is a smooth regular low mound, sometimes slightly umbonate, with a circular outline and entire margin greyish-white in color with a smooth glistening surface (Fig. 1). These colonies showed considerable variation in size, ranging from two millimetres or less up to five or six millimetres in diameter, depending upon the degree of crowding on the plate.

A second distinct colony type, the R, is strikingly different. This is a large, spreading, perfectly flat colony, frequently reaching a diameter of a centimetre and a half with a very irregular flagellated margin and a finely granular dull surface (Figs. 3 and 4). At times the outline of the colonies may become extremely irregular with feathery prolongations but with the same granular surface (Fig. 5). Occasionally the central part of the colony shows a slightly elevated area but usually this is lacking. These colonies look more like the figures of *Cl. sporogenes* and *Cl. oedematiens* (4) than classic *Cl. welchii* colonies.

In addition to these two extreme colony types, plate cultures, especially those made from old broth cultures, often exhibit a number of apparently intermediate forms. In cultures predominantly S in form colonies frequently appear with slightly serrate margins and a somewhat irregular surface, while in cultures predominantly R there may appear colonies with a smooth dome-shaped central area but with characteristic spreading, granular margins. Still other cultures produce colonies which are so nearly intermediate between



the two extreme types that classification is impossible. Subcultures of these intermediate forms generally developed S, R or mixtures of S, R and intermediate types.

In a few instances mucoid modifications of both the S and R colony forms were observed. These colonies are indistinguishable in appearance from the typical S and R forms. While the ordinary or non-mucoid S colony consists of a soft gelatinous mass readily emulsified in saline or water to form an even and highly stable suspension, the similar-appearing mucoid S colony when touched with a loop may be drawn out into mucilaginous threads one to five centimetres long. The non-mucoid R colonies though soft and gelatinous are rather more granular than the S, and emulsions, as noted in more detail later, are highly unstable. The mucoid R colonies when touched with a loop behave in the same manner as the mucoid S colonies.

### Deep Colonies

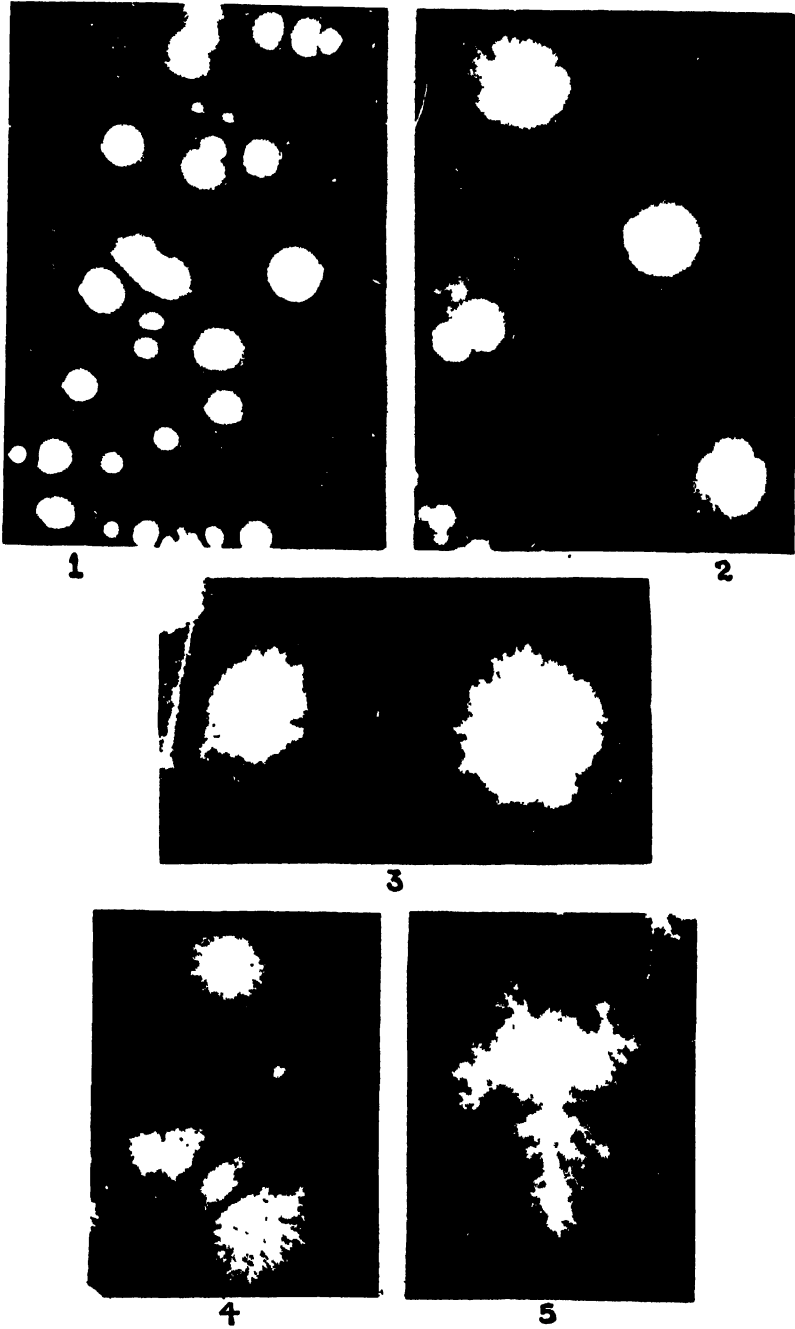
Colonies of S and R types developing below the surface of hormone agar, as in poured plates or shake agar tubes, show such differences as might be anticipated from the topography of the surface colonies. The typical S colony growing under such circumstances is a lense-shaped structure of pale milky opalescence with sharply defined margins and resembles the deep colonies of *Cl. welchii* described by Weinberg and Séguin (12). In contrast, the typical R growing as a well-isolated submerged colony appears as a loose filamentous or woolly mass.

### Morphology and Capsulation

The individual organisms of the S type are characteristic of *Cl. welchii* as ordinarily described, rather short, plump bacilli with rounded ends, generally occurring singly or occasionally in chains of two or three end to end. Staining is uniform with the ordinary dyes and definitely positive with Gram's procedure. From most culture media the organisms show capsules one to two times the thickness of the body of the bacilli. Organisms of the R type, in contrast, are much more irregular in form; generally, in films from fluid cultures, the majority are short plump bacilli like the S type, but short coccoid forms and many long bacilli, 5 to 20  $\mu$ , are to be found. Chain formation, especially in the case of the long forms, is much more common than in the case of the S type. While the S stain uniformly the R types show much granulation; with methylene blue or even with Gram's stain most organisms in a film appear to consist of a mass of fine heavily staining granules contained in an unstained matrix. Capsules, so characteristic of the S type, are almost completely absent in the R type.

### Habit of Growth in Fluids, and Cataphoresis

Well-differentiated cultures of the S type always produce in fluid media a diffuse growth in which the organisms remain uniformly suspended for long periods. Definite R cultures on the other hand produce first a diffuse followed by a bottom growth which leaves the supernatant fluid perfectly clear. It



Photographs of colonies of *Cl. welchii* growing on hormone-blood agar. FIG. 1. *S* colonies. FIG. 2. *S* colonies with very thin *R* outgrowths. FIGS. 3 AND 4. *R* colonies. FIG. 5. Widely spreading *R* colony. The photographs were made by B. Halsgrove.



has generally been found that if serial fluid cultures of an S type begin to develop bottom growth, plates will show R colonies. On the other hand when serial cultures of an R type show persistent diffuse growth in the supernatant fluid, plates will indicate the presence of S types.

This habit of growth appears to be associated with the surface charge on the organisms. As indicated in Table I, when organisms of well-differentiated S and R types were washed in the centrifuge three times and suspended in Clark's phosphate buffer solutions the R suspensions proved highly unstable, the S suspensions relatively stable: the R organisms agglutinated

TABLE I  
ACID AGGLUTINATION OF S AND R *Cl. welchii*

pH	R from C <sub>4</sub>	S from C <sub>4</sub>	R from C <sub>5</sub>	S from C <sub>5</sub>	pH	R from C <sub>4</sub>	S from C <sub>4</sub>	R from C <sub>5</sub>	S from C <sub>5</sub>
1.2	+	—	+	—	4.8	+	+	+	+
1.4	+	—	+	—	5.0	+	+	+	+
1.6	+	—	+	—	5.2	+	+	+	+
1.8	+	+	+	—	5.4	+	+	+	+
2.0	+	+	+	+	5.6	+	—	+	—
2.2	+	+	+	+	5.8	+	—	+	—
2.4	+	+	+	+	6.0	+	—	+	—
2.6	+	+	+	+	6.4	+	—	+	—
4.4	+	+	+	+	6.8	—	—	+	—
4.6	+	+	+	+	7.2	—	—	+	—

NOTE:—Washed organisms of the two types were suspended in phosphate buffer solutions; + indicates complete agglutination and precipitation, and — indicates no agglutination.

over the entire pH range tested from 2.0 to 6.4 or 7.2: the S organisms were agglutinated in mixtures with a pH range of 1.2 to 5.4 or 5.6 but remained in stable suspension in the more acid and basic mixtures. Similar suspensions tested in the Kunitz type cataphoresis apparatus exhibited isoelectric points in agreement with the acid agglutination results. Suspensions of well-established S types from various cultures were isoelectric in buffer mixtures from pH 2.0 to 2.4 with an average of pH 2.1. Suspensions of well-established R types from various cultures were isoelectric from pH 3.4 to 3.8 with an average at pH 3.6 and types showing intermediate colony forms generally exhibited intermediate isoelectric points.\*

### Origin of the Two Colony Types

The two colony types were most readily obtained by plating, as just described, from old broth or chopped-meat media cultures. On plating such old cultures for the first time usually a majority of S colonies developed but at the same time, in most instances, either a few or many flat irregular colonies of the R type also appeared. Frequently too these primary plates developed S colonies with marginal outgrowths of R-like structure, as indicated in

\*More data on the relation of cataphoresis to the variation of this species will be presented in collaboration with B. G. Gardiner.

Fig. 2. Where well-isolated and characteristic S and R colonies appeared on primary plates, pure cultures of the R form were generally obtained on the first subculture from R colonies. The R-like marginal outgrowths from S colonies likewise frequently produced pure growths of the R type though usually the first culture consisted of R and S types, and several selections were necessary before pure R cultures were obtained. In either case when this type was finally purified it exhibited a high degree of stability; after many cultural generations on this solid medium there was no apparent tendency to variation.

The S forms on the other hand, as in the case of most other species of bacteria studied from this point of view, proved to be less stable. The selection and subculture of S types in many instances resulted in the development of pure S cultures which have bred true for many generations on this solid medium. Frequently the S cultures grown from single S colony selections have continued even after many generations to produce a small proportion of S colonies with R-like outgrowths. Continued single colony selection has, however, always resulted in the development of true breeding and relatively stable S types, as long as the culture was maintained on this solid medium and propagated from single characteristic colonies.

More precise evidence of the origin of one variant type from the other has been obtained by growing carefully purified types in fluid media. In one instance a culture of strain C<sub>4</sub>, which for eight generations on solid media had exhibited only S types, was introduced into various fluid media (listed in Table II) and transferred serially at 24-hr. intervals. Under these circum-

TABLE II  
S TO R VARIATION IN *Cl. welchii*, C<sub>4</sub>

Fluid media	Colony form
9 generations in ordinary broth with 0.1% glucose	All S
4 generations in hormone broth with 0.1% glucose	All S
10 generations in hormone broth without glucose	All S
30 generations in chopped meat with 0.1% glucose	Mostly S, few R
1 generation in chopped meat with 0.1% glucose aged 8 weeks at room temperature	Mostly S, few R

NOTE:—One characteristic S colony from the eighth generation on solid media showing only S colonies was used to inoculate the fluids. Variation was determined by plating on hormone-glucose-blood agar.

stances, as indicated in Table I, the S type exhibited a high degree of stability although plates made from the 30th serial culture in a chopped-meat medium developed a few R colonies. Plates from an eight-weeks-old culture in a similar medium likewise developed a few R colonies. In both cases subcultures of the R colonies resulted in true breeding R types.

The possibility of R to S variation may be illustrated by one series in which an R type of strain C<sub>4</sub>, after producing only R colony types for eight generations on solid media, was transferred to various fluid media (listed in

Table III). On plates made from the fourth serial 24-hr. culture in ordinary broth containing 0.1% of glucose, the growth consisted of 80% R and 20% S colonies. Subcultures of several R colonies developed only R types. Sub-

TABLE III  
R TO S VARIATION IN *Cl. welchii*, C<sub>4</sub>

Fluid media	Colony form
4 generations in ordinary broth with 1% of glucose	80% R, 20% S
12 generations in hormone broth without glucose	All R
12 generations in hormone broth with 1% of glucose	All R
6 generations in chopped meat with 1% of glucose	All R

NOTE:—One characteristic R colony from the ninth generation on solid media showing only R colonies was used to inoculate the fluids. Variation was determined by plating on hormone-glucose agar.

cultures of several characteristic S colonies developed a mixture of R and S types but after two additional selections of the most characteristic S colonies a true breeding S was obtained from this strain, originally R.

### Single Cell Isolations

Results obtained with cultures purified by colony isolations were confirmed with cultures purified by single cell isolations. The methods of isolation used by Kahn (2) and by Wright and McCoy (13) were employed and the selected single cells were planted in tubes of recently boiled Robertson's chopped meat by the procedure of breaking the tips of the micropipettes below the surface of the fluid. Wright and McCoy (13) make the general statement that, in working with anaerobes, about 2% of the single cells isolated may be expected to develop. In one series with *Cl. welchii* where 50 cells were isolated from five different cultures, three (6%) grew into characteristic cultures.

An S type of C<sub>4</sub> strain which had been purified by repeated single colony isolation and which had produced only S colonies for eight successive platings on the hormone-blood agar was transferred to Robertson's chopped meat. After 24 hr. growth several single cells were isolated and placed in similar chopped-meat tubes. Three cultures developed.

These three cultures were plated in the usual manner, as just described, after 24 hr. growth in the Robertson's meat from the single cell inoculations. From the first two cultures, plates containing several hundred colonies exhibited only characteristic S forms like the parent culture. Plates from the third single cell culture developed, in addition to several hundred characteristic S colonies, two colonies showing minute R-like marginal outgrowths similar to those pictured in Fig. 3. Subcultures from these R-like outgrowths on solid media developed R and S colonies and by further colony selection pure R type cultures were obtained. It seems apparent therefore that one cultural generation in Robertson's meat from a single cell isolation may result in S to R variation.

The two remaining cultures developed from single cells were transferred to the fluid media listed in Table IV. As noted in the table, except for the series in ordinary glucose broth, all showed some S to R variation. Essentially the

TABLE IV  
S TO R VARIATION IN A CULTURE PURIFIED BY SINGLE CELL ISOLATION

Fluid media	Colony form
10 generations in ordinary broth with 0.1% of glucose	All S
8 generations in chopped meat with 0.1% of glucose in an atmosphere of 10% CO <sub>2</sub>	Mostly S, very few with R outgrowths
11 generations in chopped meat with 0.1% of glucose, 40° C.	Mostly S, few with R margins
23 generations in chopped meat with 0.1% of glucose, 37° C.	Majority S, many intermediate, many characteristic R
1 generation in chopped meat with 0.1% of glucose, 24 hr. at 37° and 2 months at 12 to 15° C.	Majority S, many intermediate, many characteristic R

NOTE:—The media were inoculated from the first generation in chopped meat from a single-cell isolation. Variation was determined by plating on hormone-glucose-blood agar.

same results were obtained with the two cultures. In both instances, by colony selection and subculturing pure R-type cultures were obtained from the original S cultures.

It is apparent therefore that similar degrees of variation of S-type organisms have been obtained from cultures purified by colony selection and by single cell isolation.

### Pathogenicity

Pathogenicity has been determined by injecting the fluid portion of Robertson's chopped-meat cultures, after 24 hr. growth, into the breast muscle of pigeons. Both the S and the R types prove to be pathogenic but the minimum killing dose of the R type is in general approximately 10 times greater than the minimum dose of S where the two types have been isolated from the same strain. Results from such tests, with a culture of relatively low pathogenicity, are indicated in Table V. In this instance 5 cc. of the R type produced approximately the same reaction as 0.5 cc. of the S type. Similar proportionate killing effects with S and R cultures from other strains have been observed.

TABLE V  
PATHOGENICITY OF S AND R TYPES OF *Cl. welchii* FOR PIGEONS, AS INDICATED BY THE KILLING ACTION OF 24-HR. CULTURES

Type	Dose, cc.	Effect
S type C <sub>4</sub>	0.1	Toxic, but birds recovered
	0.5	Death in 12 to 16 hr.; small amount of edema
	1.0	Death in 8 to 10 hr.; marked edema and necrosis
R type C <sub>4</sub>	1.0	Death in 7 to 10 days; marked edema and necrosis
	5.0	Death in 12 to 16 hr.; small amount of edema

There is now, however, considerable evidence to indicate that there is a fallacy in this method of distinguishing between the pathogenicity of the S and R types. In most instances where pigeons have been infected intramuscularly with R types and the edematous or necrotic area has been cultured after the death of the bird, R types similar to those injected, together with an appreciable percentage of S types (in some cases as high as 50% of S types), have been recovered. This has been most frequently observed where a relatively small dose of the R culture has been used so that the animals survived for several days. Since these S types are invariably highly virulent it is impossible to suppose that they were previously present in the tissue. This suggests, obviously, that the death of the animals may result from the S types arising from an R to S dissociation *in vivo*.

### Hemotoxin Production

Hemotoxin production was determined by the methods used in earlier work in this laboratory with *Cl. welchii* toxin (5, 7). The organisms were grown in the usual chopped-beef-peptone medium enriched with 0.1% of glucose as recommended by Bengston and incubated for 24 hr., the supernatant material centrifuged, filtered and the filtrate titrated immediately. The titration consisted in determining the concentration of the toxic filtrate which would produce hemolysis of a 2% suspension of washed rabbit red cells.

Toxins were prepared in this way from well-established S and R types and from the undissociated parent strains from which the dissociated types were obtained. One such titration is shown in Table VI. Both R and S

TABLE VI

TITRATION OF HEMOTOXIN PRODUCED FROM S AND R TYPES AND FROM THE PARENT STRAIN OF A CULTURE OF *Cl. welchii* WITH AND WITHOUT ANTITOXIN

	Dilutions of toxin								Cont.
	$\frac{1}{25}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	
S type, C <sub>4</sub>	+	+	+	+	+	+	+	-	-
S type, C <sub>4</sub> , plus antitoxin	-	-	-	-	-	-	-	-	-
R type, C <sub>4</sub>	+	-	-	-	-	-	-	-	-
R type, C <sub>4</sub> , plus antitoxin	-	-	-	-	-	-	-	-	-
C <sub>4</sub> original culture	+	+	+	-	-	-	-	-	-
C <sub>4</sub> original culture with antitoxin	-	-	-	-	-	-	-	-	-

types, it may be noted, produced hemotoxin but in this instance the S produced 20 times more toxin than the R and about six times more than the undissociated parent culture. Results were similar with other strains and in many preparations from the same strain.

The hemotoxin produced by both the S and R organisms was neutralized by a stock *Cl. welchii* antitoxin. In the titration results shown in Table VI a 1 to 100 dilution of a stock *Cl. welchii* horse-serum antitoxin was mixed



with the various dilutions of the toxin and incubated for half an hour before the addition of the rabbit red cells. This dilution of the antitoxin completely inhibited both the S and the R hemotoxin.

### *In vivo* Action of Hemotoxin

Much earlier work has indicated that the intravenous introduction of suitable doses of *Cl. welchii* toxin into rabbits produces a profound anemia accompanied by cellular changes which resembles pernicious anemia in man (5, 7). A particular sample of toxin recovered from an S type of culture C<sub>4</sub> in 2.5-cc. amounts produced such a change in rabbits, but a similar amount of filtrate from an R type recovered from the same culture produced negligible blood changes. The quantitative changes in red cells and hemoglobin produced by the same amounts of filtrates from S and R types and from the undissociated parent culture are shown in Table VII.

TABLE VII

THE *in vivo* ACTION OF S AND R TYPES AND THE UNDISSOCIATED PARENT CULTURE OF *Cl. welchii*

Toxin	Drop in red cells, %	Drop in hemoglobin, %
S type, 2.5 cc.	66	70
R type, 2.5 cc.	9	8.5
Undissociated, 2.5 cc	25	52

TABLE VIII

*In vivo* ACTION OF EQUAL HEMOLYTIC DOSES OF S AND R *Cl. welchii* TOXIN IN RABBITS

Toxin	Drop in red cells, %	Drop in hemoglobin, %
C <sub>4</sub> S, 2.5 cc.	66	70
C <sub>4</sub> R, 2.5 cc.	43	38

The *in vitro* action of this sample of S hemotoxin was approximately 10 times greater than the R toxin. By taking this hemolytic titre as the criterion, equal hemolytic doses of toxin were introduced intravenously into rabbits. One such result is shown in Table VIII where it is apparent that even when equal hemolytic doses are introduced, the S type toxin produces a more profound anemia than the R type.

### Antigenic Activity

Immunization of rabbits with repeated doses of heat-killed S type whole organisms results in sera which agglutinate homologous S organisms but not R organisms from the same culture. This phase of the problem will be reported in more detail in a subsequent paper.

### Species Characteristics of the Two Types

Both the S and R types behave as characteristic *Cl. welchii*. Both types produce a similar degree of stormy fermentation in milk and both produce similar fermentation reactions in those carbohydrates which have ordinarily been used in the differentiation of anaërobic bacteria. Results of such carbohydrate fermentations with S and R types isolated from three strains of *Cl. welchii* are shown in Table IX. It is apparent from the table that on the

TABLE IX  
CARBOHYDRATE FERMENTATIONS PRODUCED BY S AND R TYPES OF THREE  
STRAINS OF *Cl. welchii*

Type and strain	Milk	Glucose	Lactose	Saccharose	Galactose	Levulose	Maltose	Glycerol	Mannite	Inulin	Salicin	Dulcitol
S type, C <sub>4</sub>	Stormy	+	+	+	+	+	+	-	-	-	-	-
R type, C <sub>4</sub>	Stormy	++	++	++	++	++	++	-	-	-	-	-
S type, C <sub>5</sub>	Stormy	+	+	+	+	+	+	-	-	-	-	-
R type, C <sub>5</sub>	Stormy	+	+	+	+	+	+	-	-	-	-	-
S type, L	Stormy	+	+	+	+	+	+	-	-	-	-	-
R type, L	Stormy	+	+	+	+	+	+	-	-	-	-	-

basis of glycerol and inulin fermentation the three strains belong to *Cl. welchii* type IV, but more significant from the point of view of this investigation was the fact that the R and the S types give the same fermentations.

It is evident from these results that notwithstanding the marked differences in colony topography, pathogenicity and antigenic structure that the enzyme contents of the two types, as far as tested by these reactions, are the same.

#### Note

After this paper was completed the work of C. A. McGaughey (3) came to the writers' attention. Using a somewhat similar procedure to that just described McGaughey obtained from a culture of *Cl. welchii*, in addition to what are described as normal colony forms (apparently the same as the writers' S colonies), two variants described as the variant 1 and variant 2. Variant 1, in colony structure pathogenicity and hemotoxin production, appears to be the same as the colony form which the present writers have called R. Variant 2 resembles less closely what has been described herein as types intermediate between S and R. Mucoid variants of the normal form and variant 1, which McGaughey describes, resemble the authors' mucoid forms of S and R. It is evident, therefore, that although McGaughey's observations and the writers' are not in complete agreement the results support each other in indicating that *Cl. welchii* is subject to profound variation.

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## THE RATE OF DECOMPOSITION OF CREATINE IN ACID AND IN ALKALINE SOLUTION<sup>1</sup>

BY ALEXANDER THOMAS CAMERON<sup>2</sup> AND JEAN STIRTON GUTHRIE

### Abstract

Studies have been made on the decomposition of creatine in acid and alkaline solutions at 37.5° and 50° C. The rates of transformation to creatinine in acid solutions gave values for the Arrhenius constant varying from 20,700 to 24,000, values in moderate agreement with those of Edgar and Wakefield.

There was a definite minimal rate of transformation at approximately 0.1 *N* hydrochloric acid, and a secondary maximum at about 0.01 *N* hydrochloric acid. This was probably owing to catalysis of creatine hydrochloride and free creatine at different rates. The slightly differing values found for the Arrhenius constant in strong and in weak acid solutions may be explicable on this basis.

The results with alkali support Gaebler's views that creatine is initially transformed into urea and sarcosine, which then reunite to form ammonia and methyl hydantoic acid.

These results show further that in solutions of acid and alkali of corresponding strength, alkali catalyses the change from creatine to creatinine much more rapidly than acid.

It appeared from a recent survey of the biochemical application of the Arrhenius equation for the effect of temperature upon rate of reaction (4) that further determination of the Arrhenius constant for reactions *in vitro* that are of biochemical significance might be of some value. The transformation of creatine into creatinine was selected for initial consideration since numerous studies of this change have been recorded and the constant has already been determined by Edgar and Wakefield (7). Hence study of this change seemed very suitable for the purpose of checking accuracy of technique. The results, while largely confirming those of Edgar and Wakefield, disclosed certain facts which do not appear to have been hitherto recorded and seem to be of sufficient significance to be worth reporting. The results also lend support to Gaebler's views on the decomposition of creatine in alkaline solution.

### Experimental Technique

A preliminary series of measurements was made with 0.5% commercial creatine hydrate and different strengths of hydrochloric acid and sodium hydroxide, at temperatures of 37.5° and 50° C. The concentration of creatine was selected as being almost the strongest possible for the method employed, and as corresponding roughly to the average creatine content of striped muscle. These preliminary measurements were of great value in indicating approximate results, since they permitted, in the subsequent accurate series, colorimetric comparison between alkaline creatinine picrate solutions of almost equal strengths.

For the accurate series a good commercial creatine was recrystallized once from hot water. Attempts to air-dry this to give the pure hydrate failed

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since, in midwinter in Winnipeg, air-drying caused a slow loss of water from the hydrate. Hence the preparation was dried to constant weight (six days) at 105° C. It analyzed (mean of four determinations) 32.04% nitrogen (by Kjeldahl) as compared with the theoretical value 32.06%. A test in which 0.44 gm. was extracted with *cold* water at room temperature showed, by colorimetric procedure, no detectable trace of creatinine.

In the preliminary series commercial creatinine was used for colorimetric comparison. For the accurate series a good commercial creatinine zinc chloride was recrystallized from hot water and air-dried for three days. It then contained 0.3% of water; further air-drying did not alter this content. It was therefore used and corrected for this trace of moisture, 1.608 gm. per litre being taken as equal to 0.1% of creatinine. It analyzed (corrected for water content) 22.80% nitrogen (mean of three determinations; theory 23.18%) and 19.35% chlorine (mean of two determinations; theory 19.57%).

Twice-distilled water was employed for all creatine and creatinine solutions and for dilution of all acid and alkaline solutions. Mallinckrodt c.p. hydrochloric acid and Kahlbaum (alcohol-purified) sodium hydroxide were used; the oxalic acid used for standardization was a B.D.H. A.R. preparation. Titration values for acid and alkaline solutions indicated an accuracy almost always within 0.2%.

In the accurate series the temperatures of the water bath were 37.5° and 50.2° C., both  $\pm 0.1^\circ$  and usually  $\pm 0.05^\circ$  (standardized thermometers). Standardized pipettes, burettes, and flasks were invariably employed.

For each experiment an accurately weighed amount of creatine was dissolved in water rapidly with application of slight heat. Equal volumes of the solution and of acid or alkali of suitable strength were pipetted, one into a wide-mouthed 500-cc. bottle, and another into a tall weighing bottle introduced within it. The large bottle was then closed with a rubber cork and almost completely immersed in the thermostat. When its contents had attained the temperature of the bath (30 or 40 min.) the weighing bottle was overturned with a glass rod, immediate shaking producing an accurate zero time. The mixed solution of course contained creatine and acid or alkali of strength half that prior to mixing. At suitable time intervals an exact amount of solution was pipetted into an equal volume of alkali or acid of strength corresponding to that of the mixed solution, thus producing immediate neutralization and stopping the reaction. Five cubic centimetres was usually used, 2 cc. when the amount of creatinine was large.

### *Estimation of Creatinine*

The usual Folin colorimetric procedure was slightly modified. To the 10-cc. neutralized sample was added 10 cc. of alkaline picrate. The approximately correct amount of suitable standard was measured from a burette and distilled water added from a second to total 10 cc., and to this 10 cc. of alkaline picrate was also added. After five minutes comparisons were made rapidly in a micro-colorimeter, using artificial illumination.

Two standards were employed, one containing 0.1% creatinine, the other 0.02%. In estimating very dilute solutions of creatinine (less than 0.4 mgm. of creatinine in the 5-cc. sample) it was found necessary to add a measured amount of standard to the unknown. The subsequent correction of course increased the possible error. The lengths of columns of standard were adjusted to suitable depth of color. Solutions containing more than 3 mgm. of creatinine in the original 5-cc. sample required further dilution with water to an extent depending on depth of color. Throughout, parallel dilution procedures were used with unknown and standard.

The alkaline picrate was made up daily in the ratio of 100 cc. of saturated solution of picric acid to 20 cc. of exactly 10% sodium hydroxide. Tests showed that such alkaline solutions 18 hr. old gave perfect correspondence with those freshly prepared. The picric acid used was purified by the procedure of Halverson and Bergeim (cf. Hawk and Bergeim (12)).

In making the actual colorimetric reading the standard was placed in both cups, the light incidence and the cup level on one side adjusted to perfect color match, and the solution in that cup replaced by the unknown and four readings taken, the mean of these being used.

*Factors affecting the probable error of the creatinine estimation.* Difference in degree of alkalinity between standard and unknown picrates to the extent of 0.01 *N* gave no error, to the extent of 0.025 *N*, no definite error, but to the extent of 0.05 *N* gave a marked difference (about 17%). Addition of sodium chloride to give a concentration of 1.8% did not affect the standard. This was equivalent to the amount formed in neutralizing the stronger acid and alkaline solutions.

Comparison of two known solutions of relative strengths 4 : 5 gave results differing by 0.5% or less from accuracy. Few of the comparisons made in the accurate series exceeded this ratio.

Two important sources of error were found. The creatinine zinc chloride standards employed definitely deteriorated with time. The error for the stronger standard was negligible for three weeks, but thereafter the strength decreased with increasing rapidity. The weaker standard deteriorated slightly more rapidly. In a few of the earlier experiments corrections had to be applied for this error.

Application of heat to bring creatine into solution involves a slight conversion to creatinine, the amount formed varying with the temperature. Hence in every case a zero determination was necessary in the creatine solution. The usual correction was found to be about 2 mgm. per 100 cc.; obviously where the rate of change is small the exactitude of this correction is important. In aqueous solutions of creatine that were being heated in the water bath to the experimental temperature (before mixing with acid or alkali) the error due to this application of heat was not measurable at 37.5° but was just definite at 50° C. Hence for experiments at the latter temperature the zero correction was determined by immersing the creatine solution at 50° C. for 40 min. before estimation.

It is impossible to determine the probable error of the creatinine determinations by statistical methods, since it varied chiefly with the degree of fatigue of the eye. Slight fatigue appeared to be desirable, apparently due to an enhancement effect (cf. Allen, 1, 2, 3). Experience showed that only one accurate set of readings of the reddish-orange colors could be made in each half hour, and after several hours of such half-hourly readings fatigue effect was evident through decreasing agreement in consecutive readings. Moreover, experiments over several consecutive days showed decreasing accuracy from day to day.

Error from unequal illumination is marked but avoidable.

In spite of the large potential fatigue error the agreement that was actually obtained for like conditions in different experiments suggests that the mean of three experiments was almost always within 2% of accuracy, if not within 1%.

*Estimation of total creatinine.* In the few experiments (with alkaline solutions) in which such estimations were made, since large amounts had to be measured, 5-cc. samples were taken, exactly neutralized with 5 cc. of acid, then 10 cc. of 2 *N* hydrochloric acid added and the mixture autoclaved for 30 min. at 18 to 20 lb. pressure. After cooling 10 cc. of 2 *N* sodium hydroxide and 25 cc. of alkaline picrate were added, and after five minutes the whole was diluted to 250 cc. and compared with a standard of similar strength of creatinine.

*Estimation of urea in alkaline creatine solutions.* The usual sampling procedure was employed, but since each sampling permitted loss of ammonia from the solutions duplicate 10-cc. samples were removed at each period, and immediately neutralized. The ammonia evolved from one sample, after addition of alkali, was carried over with a stream of air into *N*/50 acid for pre-formed ammonia, and the other was decomposed with urease and then similarly treated, the difference giving the true urea value. An error of one drop of *N*/50 alkali in the final titration corresponded to 0.03 mgm. of urea or 0.07 mgm. of creatine per 100 cc. of solution. Subtraction of the ammonia value doubles the possible error from this source.

*Estimation of ammonia in alkaline creatine solutions.* Since much of the ammonia passes to the gas phase consecutive sampling could not be employed. Immediately after mixing creatine and alkali, 10-cc. samples were transferred to each of eight thick-walled urea tubes already at the experimental temperature and these were tightly stoppered. One was used for a zero determination, the others removed at definite time intervals, the ammonia aerated into *N*/50 acid and this titrated against alkali. The error was of an order corresponding to that for urea.

### Experimental Results

Table I illustrates the degree of agreement (and non-agreement) in the results of a single series. The figures give the number of mgm. of creatinine produced in 100 cc. of a solution of 0.4396% creatine (equal to 0.5% creatine hydrate) and 0.1 *N* hydrochloric acid at 37.5° C.

TABLE I  
A TYPICAL SERIES

Time, hr.	Experiment No.								Mean
	1	2	3	4	5	6	7	8	
0.5	4.6	4.5	—	—	4.6	—	—	—	4.6
1.0	—	8.9	8.5	8.6	—	—	—	—	8.7
1.5	—	—	—	12.2	12.4	—	—	12.8	12.5
2.0	15.2	15.7	—	—	—	15.5	—	—	15.5
2.5	—	—	—	—	—	18.7	18.6	18.8	18.7
3.0	—	—	—	—	22.7	22.3	—	22.9	22.6
3.5	—	—	—	27.4	—	28.8	—	28.2	28.1
4.0	—	33.4	32.6	32.8	—	—	—	—	32.9
5.0	—	—	—	—	—	40.8	40.9	41.2	41.0
6.0	—	48.9	49.3	—	—	48.5	—	—	48.9
7.0	—	—	55.7	—	—	—	—	—	(55.7)
8.0	—	63.1	—	—	—	—	—	—	(63.1)

NOTE:—Creatine, 0.4396%; hydrochloric acid, N/1; temp., 37.5° C.

The results for different strengths of acid at 37.5° are shown in Table II, and at 50.2° C. in Table III, the concentration of creatine being always 0.4396%. Table IV presents results in acid solution for different creatine concentrations. All figures are in mgm. of creatinine per 100 cc. of solution. Those asterisked are for single determinations; the others are almost all the means of three determinations.

Table V gives the results for creatinine, total creatinine, urea, and ammonia in solutions containing 0.4396% of creatine and 0.1 N sodium hydroxide at 50.2° C., all figures being in mgm. per 100 cc.

TABLE II  
CREATININE PRODUCED IN ACID SOLUTION

Time, hr.	Normality of hydrochloric acid								
	2.0	1.0	0.33	0.10	0.033	0.010	0.0033	0.0010	0.0001
0.5	12.4	4.6	—	—	—	—	—	—	—
1.0	22.4	8.7	2.2*	1.1*	2.1*	4.1	4.4*	1.7*	—
1.5	28.7	12.5	—	—	—	—	—	—	—
2.0	40.2	15.5	4.4*	1.9*	—	8.5	6.8*	—	—
2.5	50.8	18.7	—	—	—	—	—	—	—
3.0	59.0*	22.6	—	—	6.2*	12.6	—	5.1*	—
3.5	65.3	28.1	—	—	—	—	—	—	—
4.0	—	32.9	8.8*	—	—	16.3	10.8*	—	—
4.5	—	—	—	3.0*	—	—	—	—	—
5.0	87.3	41.0	9.5*	—	8.2*	18.9	—	7.3*	—
5.5	—	—	—	—	—	—	—	—	1.3*
6.0	99.9	48.9	12.2*	3.9*	—	22.6	17.4*	—	—
7.0	—	55.7*	—	—	—	24.5*	—	9.4*	—
8.0	—	63.1*	—	4.1*	—	—	—	—	—
24.0	—	—	—	13.1*	—	79.0*	—	19.6*	—

NOTE:—Creatine, 0.4396%; temp., 37.5° C.

TABLE III  
CREATININE PRODUCED IN ACID SOLUTION

Time, hr.	Normality of hydrochloric acid					
	2.0	1.0	0.33	0.10	0.033	0.010
0.25	21.7	9.6	—	—	—	—
0.50	44.1	17.6	—	—	—	—
0.75	66.3	27.6	—	—	—	—
1.00	86.8	32.8	7.4*	1.5*	5.1*	15.1
1.25	116.8	45.9	—	—	—	—
1.50	—	52.6	—	—	—	—
2.0	161.4	66.8	15.4*	—	—	29.8
2.5	—	—	—	—	—	37.4*
3.0	233.7	92.2	—	6.1*	—	44.3
3.5	—	—	—	—	—	50.5
4.0	—	119.6	—	—	22.5*	57.4
5.0	—	147.2	—	—	—	66.9
6.0	—	—	—	—	—	81.0
7.0	—	—	—	15.4*	—	92.7
8.0	—	—	—	—	—	115.8

NOTE:—Creatine, 0.4396%; temp., 50.2° C.

TABLE IV  
RATE OF CREATININE PRODUCTION FOR VARYING CONCENTRATION OF CREATINE

Conc. creatine, %	Normality HCl	Time, hr.		
		1	2	3
0.4396	1.0	32.8	66.8	92.2
0.2198	1.0	14.7*	28.5*	42.0*
0.1099	1.0	8.4*	14.4*	20.9*
0.4396	0.01	15.1	29.8	44.3
0.2198	0.01	6.5*	12.6*	20.0*
0.1099	0.01	2.6*	5.3*	7.3*

NOTE:—Temp., 50.2° C.

TABLE V  
ESTIMATION OF DECOMPOSITION PRODUCTS OF CREATINE IN ALKALINE SOLUTION

Time, hr.	Creatinine	Total creatinine	Urea	NH <sub>3</sub>
0.5	118	353*	—	—
1.0	137	346*	8.0*	0.9
1.5	144	—	—	—
2.0	146	328*	14.0	1.8
2.5	142	—	—	—
3.0	136	318*	20.3*	3.2
4.0	120	304*	26.0	4.0
4.5	117	—	—	—
5.0	115	300*	31.2	4.9
5.5	110	—	—	—
6.0	104	—	33.5*	6.3
7.0	101	—	41.2*	7.1
8.0	99	—	42.5*	—

NOTE:—Creatine, 0.4396%; sodium hydroxide, N/1; temp., 50.2° C.



## Discussion of Results

Examination of Tables II and III reveals a definite minimum rate of creatinine formation for 0.1 *N* acid; with decreasing strength of acid a secondary maximum occurs at about 0.01 *N* hydrochloric acid. This is well shown by the figures for a 24-hr. period. The data in the two tables are presented graphically in Figs. 1 and 2.

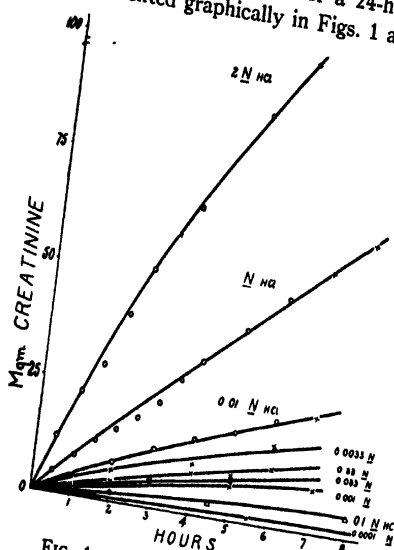


FIG. 1. Curves illustrating the relative rates of transformation of a 0.4396% solution of creatinine into creatinine in different concentrations of hydrochloric acid at 37.5° C. Results in mgm. creatinine per 100 cc. solution. (cf. Table II).

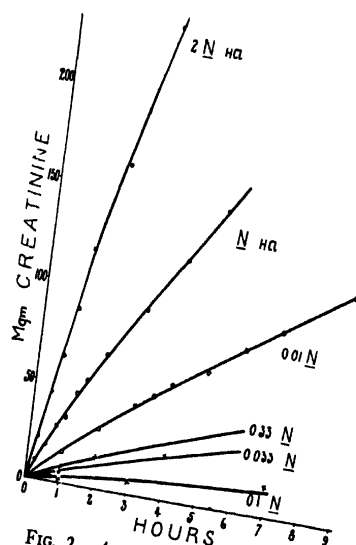


FIG. 2. As in Fig. 1, but at 50.2° C. (cf. Table III).

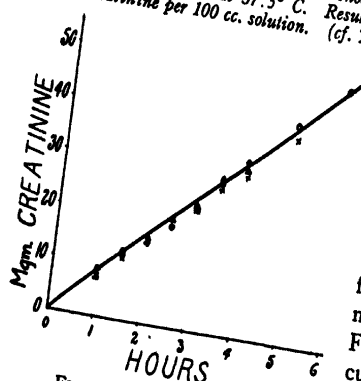


FIG. 3. Results of three observers on the relative rates of transformation of a 0.4396% solution of creatinine into creatinine in *N* hydrochloric acid at 37.5° C. (Circles represent the mean of three readings by the same observer, crosses and triangles, single readings.)

(The non-fit of the curve between the 2nd and 4th hours for *N* hydrochloric acid (Fig. 1) appears to be due to experimental error. Fig. 3, recording the readings of three different observers for this strength of acid, does not indicate any definite break in the curve.)

Certain results in the preliminary series, from experiments at 99° C., confirm the minimum observed for 0.1 *N* acid (see Fig. 4); the actual figures from which the curves were drawn require substantial correction for the effect of such a temperature on neutral creatine solutions.

The presence of a minimum rate for 0.1 *N* hydrochloric acid, and a secondary maximum for 0.01 *N*, is illustrated more clearly if the amount of creatinine produced in a definite

time be plotted against the logarithm of the normality of the acid. This has been done for one- and two-hour intervals in Figs. 5 and 6.

The simplest explanation of the apparent anomaly would appear to be that there are two reactions proceeding simultaneously, one of which is much more susceptible to the catalytic influence of the hydrogen ion than is the other. In the presence of hydrochloric acid, creatine hydrochloride or its ionized components are produced. In the presence of excess of acid the transformation is mainly that of the (ionized or unionized) hydrochloride, but when creatine is in marked excess the action must be mainly on creatine itself. The latter is apparently much more susceptible to dehydration than the hydrochloride, since  $N/3$  and  $N/100$  acid solutions give comparable results. If such a theory be accepted, then decreasing concentration of both hydrochloride and hydrogen ions seems to permit the possibility of that minimum rate which actually occurs with  $0.1\ N$  acid.

Accurate determinations of the actual hydrogen ion concentrations in such solutions should determine the soundness of this view. Colorimetric comparisons have been made of the pH in pure acid solutions, and those containing  $0.44\%$  of creatine. They are admittedly not very accurate, yet they lend some support. The results are shown in Table VI and also the molar ratios of the solutions employed throughout the experiments;  $0.44\%$  creatine is approximately  $0.033$  molar.

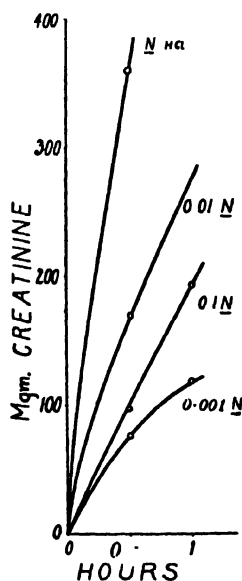


FIG. 4. Curves illustrating the relative rates of transformation of a  $0.4396\%$  solution of creatine into creatinine in different strengths of hydrochloric acid at  $99^\circ\text{C}$ . (uncorrected results in mgm. creatinine per 100 cc. solution).

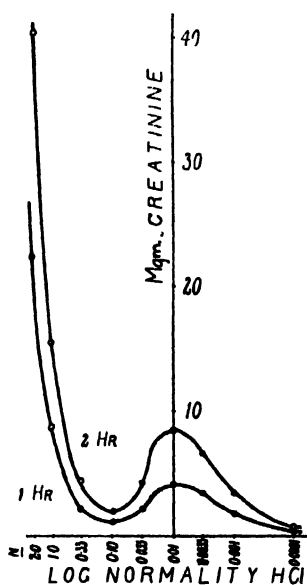


FIG. 5. Amounts of creatinine (in mgm. per 100 cc. solution) produced in one and in two hours from a  $0.4396\%$  creatine solution in different strengths of hydrochloric acid at  $37.5^\circ\text{C}$ ., plotted against the logarithmic values of the normality of the acid.

The anomalous results should, if the explanation offered be correct, have a direct bearing on much of the previous work dealing with the velocity constants of this reaction.

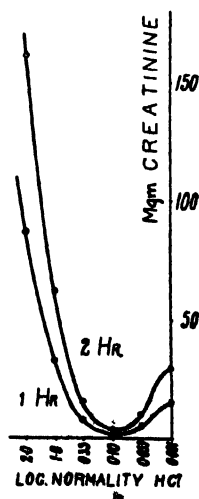


FIG. 6. As in Fig. 5, but at  $50.2^\circ\text{C}$ .

TABLE VI

MOLAR COMPARISON OF CREATINE AND ACID AND CERTAIN pH VALUES

Normality of HCl	Molar ratio creatine : HCl	Creatinine formed in 1 hr.		Probable main reaction: dehydration of	pH		(H <sup>+</sup> )		Molar concentration in creatine-HCl solutions	
		37.5° C.	50.2° C.		Pure HCl	HCl + creatine	Pure HCl	HCl + creatine	Creatine hydrochloride	Free creatine
2.0	1 : 60	22.4	86.8	Creatine-HCl	—	—	—	—	—	—
1.0	1 : 30	8.7	32.8	Creatine-HCl	—	—	—	—	—	—
0.33	1 : 10	2.2	7.4	Creatine-HCl	—	—	—	—	—	—
0.10	1 : 3	1.1	1.5	Creatine-HCl	1.0	1.2	0.1	0.06	0.033	0.000
0.033	1 : 1	2.1	5.1	Creatine	1.5	2.2	0.03	0.006	0.024	0.009
0.010	3.3 : 1	4.1	15.1	Creatine	2.0	3.0	0.01	0.001	0.009	0.024
0.0033	10 : 1	(4.1)	—	Creatine	2.7	3.5	0.002	0.0003	0.0017	0.0313
0.0010	33 : 1	1.7	—	Creatine	3.0	4.2	0.001	0.00006	0.0009	0.0321
0.0001	333 : 1	(0.3)	—	Creatine	4.4	5.7	0.00004	0.000003	0.00004	0.03296

The validity of such work would appear to be rendered questionable for concentrations of acid above 0.01 *N* (cf. Hunter (13)).\*

Figs. 1, 2, and 7 indicate that with increasing concentration of acid (above 0.1 *N*) or of creatine there is a relatively greater increase in rate of reaction, results in accordance with usual findings for such reactions.

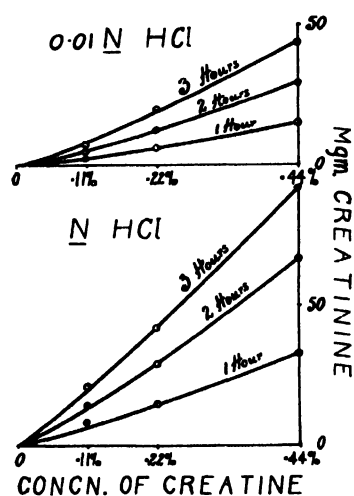


FIG. 7. Curves illustrating the relatively greater increase in speed of transformation, with increasing concentration of creatine. Results in mgm. creatinine per 100 cc. solution.

\*According to Hahn and Fasold (9), there is a somewhat similar complexity in alkaline solutions of creatine. The alkaline salts which are formed hydrolyze much more slowly than does the free creatine. Figs. 8 and 9 also suggest such complexity.

†See Cameron (4, p. 59) for a discussion of the significance of the form of expressing  $\mu$ .

For the determination of the Arrhenius constant accurate series of determinations are available for 2 *N*, *N*, and 0.01 *N* hydrochloric acid. Table VII shows that the results are in reasonable agreement with those for a monomolecular reaction. The Arrhenius constant is given by the equation

$$\mu = 4.6 \times \frac{(\log k_2 - \log k_1)}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where  $k_1$  and  $k_2$  are the velocity constants at the absolute temperatures  $T_1$  and  $T_2$ .

The summarized figures for the constant for 0.4396% of creatine and the temperatures 37.5° and 50.2° C. are as follows:—for 2 *N* hydrochloric acid,  $\mu$  equals  $24.0 \times 10^3$ ; for *N* acid,  $23.2 \times 10^3$ ; and for 0.01 *N* acid,  $20.7 \times 10^3$ .†

Edgar and Wakefield's figures (7), with varying concentrations of creatine, are as follows:—for 25° and 57° C. and 0.38 *N* hydro-

TABLE VII

REACTION VELOCITIES AND ARRHENIUS CONSTANTS FOR THE CREATINE-CREATININE REACTION

Time, hr.	2 N Hydrochloric acid				N Hydrochloric acid				0.01 N Hydrochloric acid			
	37.5° C.		50.2° C.		37.5° C.		50.2° C.		37.5° C.		50.2° C.	
	Resi- dual crea- tine	k	Resi- dual crea- tine	k	Resi- dual crea- tine	k	Resi- dual crea- tine	k	Resi- dual crea- tine	k	Resi- dual crea- tine	k
0.25	—	—	414.4	.1026	—	—	428.5	.0444	—	—	—	—
0.50	425.2	.0289	388.4	.1076	434.3	.0105	419.2	.0413	—	—	—	—
0.75	—	—	366.2	.1058	—	—	407.6	.0437	—	—	—	—
1.00	413.6	.0265	338.9	.1130	429.5	.0101	401.6	.0393	434.8	.00477	422.1	.0176
1.25	—	—	304.1	.1280	—	—	386.4	.0448	—	—	—	—
1.5	406.3	.0228	—	—	425.1	.0097	378.6	.0432	—	—	—	—
2.0	393.0	.0243	252.4	.1205	421.6	.0091	362.1	.0421	429.7	.00494	405.0	.0178
2.5	380.7	.0250	—	—	417.9	.0088	—	—	—	—	—	—
3.0	371.2*	.0245	168.5	.1388†	413.4	.0089	332.6	.0404	425.0	.00489	388.2	.0180
3.5	363.9	.0234	—	—	407.0	.0096	—	—	—	—	381.0	.0178
4.0	—	—	—	—	401.4	.0099	300.9	.0412	420.7	.00477	373.0	.0178
5.0	338.3	.0227	—	—	392.0	.0099	268.8	.0427	417.7	.00444	362.0	.0169
6.0	323.7	.0221	—	—	382.9	.0100	—	—	413.4	.00447	345.6	.0174
7.0	—	—	—	—	375.0*	.0099	—	—	411.2*	.00414†	332.1	.0174
8.0	—	—	—	—	366.4*	.0099	—	—	—	—	—	—
9.0	—	—	—	—	—	—	—	—	—	—	305.3	.0176
	Mean	.0245	Mean	.1129	Mean	.0097	Mean	.0423	Mean	.00471	Mean	.0176
	$\mu = 24.0 \times 10^3$				$\mu = 23.2 \times 10^3$				$\mu = 20.7 \times 10^3$			

\*Single determination.

†Excluded in determination of mean.

chloric acid,  $\mu$  equals  $19.05 \times 10^3$ ; for 57° and 78° C. and 0.38 N acid,  $20.4 \times 10^3$ ; for 78° and 100° C. and 0.38 N acid,  $20.5 \times 10^3$ ; and for 78° and 100° C. and 0.19 N acid,  $19.8 \times 10^3$ .

The data presented are scarcely sufficient to stress the differences obtained with weak and with strong acid, although such differences may well be real, indicating different constants for the dehydration of creatine hydrochloride and of creatine itself.

Since the conversion of creatine into creatinine is catalyzed by both hydrogen and hydroxyl ions theoretically the reaction affords an opportunity of contrasting the Arrhenius constants for these two agents under similar conditions. Preliminary determinations showed, however, that a prolonged study of the complex reaction in alkaline solution will be essential before such a contrast can be made. These preliminary studies are recorded in Figs. 8 and 9 (in which the continuous lines represent subsequent accurate determinations). Some results bearing upon the general decomposition of creatine in alkaline solution will be recorded.

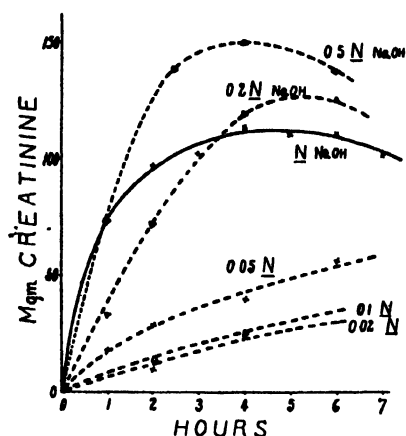


FIG. 8. Curves illustrating preliminary studies of the rate of transformation of a 0.4396% creatine solution in different strengths of sodium hydroxide at 37.5° C. Results in mgm. creatinine per 100 cc. solution.

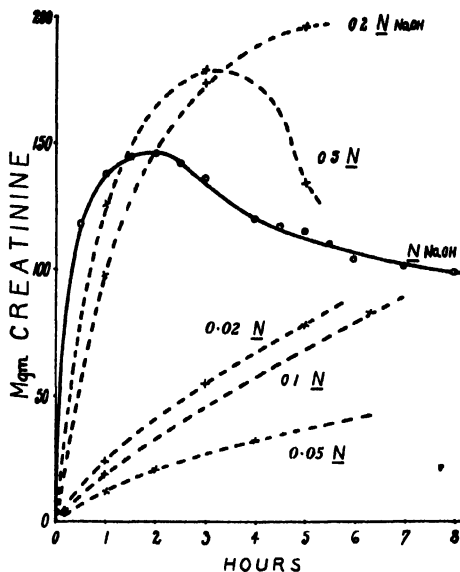
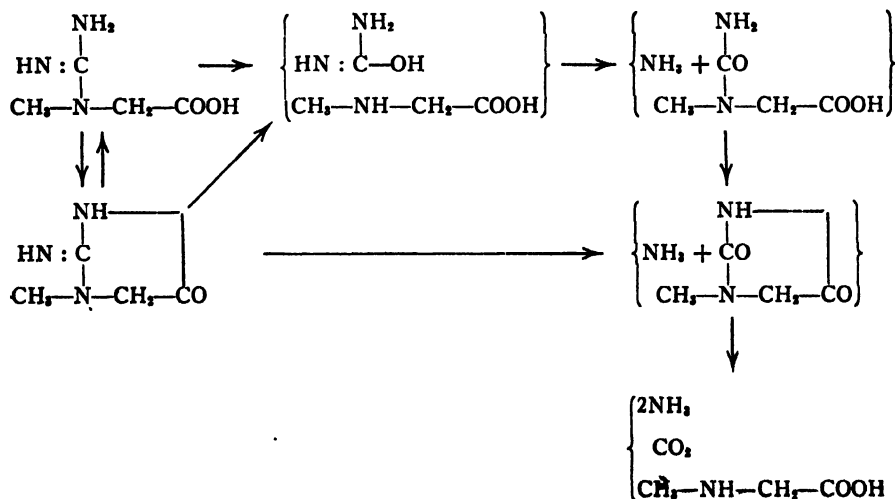


FIG. 9. As in Fig. 8, but at 50.2° C.

According to Gaebler (8) the initial decomposition of creatinine (or of creatine) by alkali is into urea and sarcosine, which then reunite to form ammonia and methyl hydantoic acid, methyl hydantoin being subsequently formed. (Collip's isolation of methyl hydantoin from testicular material may, from this point of view, indicate that it is formed in the organism from creatine, in which testicular material is relatively rich.) In accordance with Gaebler's views, the decomposition of creatine may be represented by the scheme:



From the results in Table V (and their graphical representation in Fig. 10) it is evident that the formation of ammonia is much slower than that of urea, in support of Gaebler's views. These results also indicate that the apparently rapid slowing down of creatinine formation, shown in the curves of Figs. 8 and 9, is but little due to the formation of urea and sarcosine (and ammonia and methyl hydantoic acid), but is mainly to be attributed to the relatively more rapid conversion of creatinine into creatine. Bearing this in mind, the results exemplify a point that is seldom stressed, that in solutions of acid and of alkali of corresponding strength the alkali catalyzes the change from creatine to creatinine much more rapidly than does the acid.

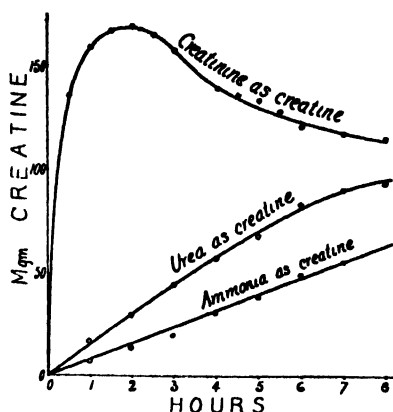


FIG. 10. Curves illustrating the relative rates of formation of creatinine, urea, and ammonia in a 0.4396% solution of creatine, and in *N* sodium hydroxide solution, at 50.2° C. All results are expressed in terms of creatine and per 100 cc. solution.

When the results are presented (Table VIII) in such form that all figures for creatinine, for urea (representing the urea and sarcosine stage), and for ammonia (representing the final stages), are expressed in terms of creatine, the total agrees within the limit of error with the original amount of creatine, so that any changes not included in the above scheme of reactions can occur only to a negligibly small extent. Fig. 11 therefore summarizes in graphical form the reaction in alkaline solution for the conditions stated.

TABLE VIII  
SUMMATION OF RESULTS OF ACTION OF *N*/1 NaOH ON CREATINE AT 50.2° C.

Time, hr.	0.5	1	2	3	4	5	6	7
Residual creatine	274	243	211	212	214	215	(198)	(178)
Creatinine as creatine	136	159	169	157	139	133	120	117
Urea as creatine	9*	17	30	44	57	68	73	90
Ammonia as creatine	4*	7	14	25	31	38	49	55
Total as creatine	423	426	424	438	441	454	—	—
Total creatine used	440	440	440	440	440	440	440	440
Error	-17	-14	-16	- 2	+ 1	+14	—	—
Error, %	-3.9	-3.2	-3.6	-0.5	+0.2	+3.2	—	—

\*Calculated from curves.

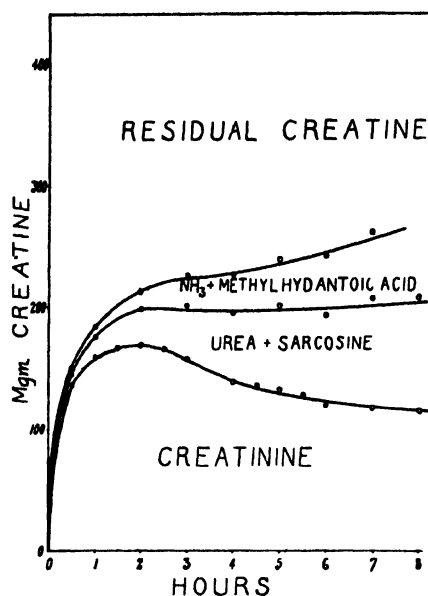


FIG. 11. Scheme summarizing the transformation of creatine (0.4396% solution) in *N* sodium hydroxide solution at 50°C. Results calculated in terms of creatine and per 100 cc. solution.

The results of Cannan and Shore (5) and those of Hahn and Meyer (10, 11) appeared to give a satisfactory explanation for the conversion of creatine into creatinine in muscle, provided muscle creatine were free. The discovery of phosphocreatine has rendered such calculations invalid. The present results are insufficient to permit definite conclusions but give a slight indication that even at the hydrogen ion concentration in fatigued muscle the rate of formation of creatinine (as indicated by its rate of excretion), if due simply to catalysis, by hydrogen ions, of such free creatine as is transiently present, is insufficient to account for the amount actually excreted.

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## THE INFLUENCE OF ELECTROLYTES ON THE FORMATION AND DECOMPOSITION OF URATE GELS<sup>1</sup>

BY E. GORDON YOUNG<sup>2</sup>, F. F. MUSGRAVE<sup>3</sup> AND H. C. GRAHAM<sup>3</sup>

### Abstract

A study has been made of the influence of various electrolytes in promoting gelation of aqueous supersaturated solutions of the urates of methylamine, lithium, piperazine and tetramethylammonium. The cation has been found to be the active agent. The minimal concentration of the electrolyte which is effective has been determined over the gelation range of the urate. The order of efficacy of the cations may be stated in general to be  $K > NH_4 > Rb > Cs > Li > Na$ . A maximal concentration of electrolyte has been found above which immediate precipitation rather than gelation occurs. The time required for gelation varies inversely with the amount of electrolyte present in any single concentration of urate. The rigidity varies directly up to the point of precipitation. The time of crystallization of the gel varies *directly* with the concentration of the electrolytes, sodium chloride, rubidium chloride and lithium chloride but *inversely* with potassium chloride and ammonium chloride. Certain organic diamines have been found to possess the power of causing gelation over a limited range of urate. Ethyl alcohol, between 20 and 60%, causes gelation of lithium urate and methylamine urate.

The thixotropic effect has been observed in gels of piperazine urate containing potassium chloride and lithium urate with alcohol.

The action of electrolytes on urate gels is interpreted as partially electrokinetic and partially lyotropic in nature.

It has been observed by Schade and Boden (9) that supersaturated solutions of sodium urate may be aided in forming gels by the addition of concentrated solutions of certain common salts. They have used sodium and potassium chlorides, ammonium, magnesium and sodium sulphates and trisodium phosphate. While investigating the influence of physical and chemical conditions on the preparation of various urates as gels the importance of the presence of electrolytes was confirmed in this laboratory (12). Two classes of urates were found; one spontaneously forming gels on cooling, a temperature-gelation effect; the other forming gels only in the presence of electrolytes, an electrolyte-gelation effect. Because of the general distribution of electrolytes in biological material, the possible importance of this effect in nature is apparent. Particularly is this so in the light of the occurrence and unusual behavior of abnormally high concentrations of uric acid in blood and urine (3, 8, 11). A more detailed study of this effect was therefore undertaken using two examples of urates which form gels spontaneously, methylamine and lithium urates, and two which do not, tetramethylammonium and piperazine urates.

The general plan of the investigation has been to study the influence of various concentrations of electrolyte on supersaturated solutions of urate below the concentration at which gels form spontaneously. The minimal

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value of the electrolyte which would induce a thin gel at a particular concentration of urate has been noted for comparative purposes. In other series the maximal value allowing gels to form has been observed also. Finally the influence of certain non-electrolytes which behave as dehydrating agents has been determined in an effort to explain the mode of action of the electrolyte in gel formation.

## Experimental

### *Methylamine Urate*

Methylamine was selected as the first base with which to make urate preparations because of the ease with which supersaturated solutions were formed and the short duration of the gel state before crystallization took place. The limits of concentration forming gels have been determined by the writers to be 1.5–10%, and the time required for crystallization from a few minutes up to 24 hr. at 20° C. depending upon concentration.

In the earlier experiments a definite concentration below that at which gels would form in pure solution was adopted and the effect of a large number of electrolytes observed. In the later experiments the range of concentrations of urate which could be induced to gel by electrolytes was determined using a small selected group of salts.

The procedure was to suspend 0.3 gm. of pure crystalline uric acid in 50 cc. of distilled water previously heated to about 90° C., to add a few drops of phenol red (0.02%), and an aqueous solution of methylamine (33%) drop by drop until the uric acid had dissolved completely and the pH was approximately 7. This clear solution was rapidly divided into fractions of 10 cc. each, varying quantities of a normal solution of electrolyte were added and the tubes cooled in an ice bath. The criterion of gel formation was judged by the rigidity of the solution on placing the test tube horizontally. This is very approximately equivalent to accepting a particular viscosity as end point to be attained in all preparations. More exact technique would be difficult because of the nature of urate gels and their non-elasticity.

The conditions adopted were the result of previous experience. It is essential that the pH be carefully adjusted because, at the lower concentrations of methylamine urate, gelation will readily take place only in the narrow range, 6.8–7.3. In such cases where the electrolyte added was either acidic or basic, allowance had to be made for this in the initial pH so that the final pH of the solution would be approximately 7. During the formation of the gel the pH shift to the alkaline side is observable as previously recorded (12).

At a concentration of 0.6% uric acid the following salts of potassium were found to induce gelation,—iodide, chloride, nitrate, chromate, sulphate, primary and secondary phosphates; of ammonium, the chloride, nitrate, chromate, sulphate, primary and secondary phosphates. The writers have been unsuccessful in causing gelation in the presence of salts of potassium or ammonium with weak acid radicals such as arsenate, arsenite, citrate or borate. Tertiary phosphates are difficult to use because of their basicity and

influence on the final pH. Salts of the heavier metals such as barium, calcium, magnesium and aluminium, caused immediate precipitation presumably of the respective urate.

In Table I are recorded as millimoles per litre the effective salts of potassium and ammonium in the minimal concentration at which they have caused gelation of methylamine urate in 0.6% solution. The time required for setting is also listed although this varies considerably and depends upon the amount of electrolyte present. The "potassium" gels were more stable than the "ammonium" gels. It is apparent from Table I that there is no difference in the efficacy of the anions Cl, I, NO<sub>3</sub>, SO<sub>4</sub>, CrO<sub>4</sub>, PO<sub>4</sub>. From the concentrations of electrolyte required it may be concluded that the active ion is the cation and presumably the urate micelle is negatively charged. This is in agreement with the conclusion of Keeser and Zocher (4) from different experimental data.

TABLE I  
GELATION OF METHYLAMINE URATE

Electrolyte	Minimal conc., millimoles/litre	Time of gelation, min.	Electrolyte	Minimal conc., millimoles/litre	Time of gelation, min.
Ammonium chloride	25	20	Potassium chloride	25	30
Ammonium nitrate	25	15	Potassium nitrate	25	180
Ammonium sulphate	12	25	Potassium sulphate	12	120
Ammonium chromate	12	90	Potassium chromate	12	300
Ammonium phosphate (primary)	8	120	Potassium phosphate (primary)	8	120
Ammonium phosphate (secondary)	7	240	Potassium phosphate (secondary)	8	120
Potassium iodide	25	60			

Selecting the chlorides as suitable salts a study was next made of the concentration of electrolyte required to cause gelation in decreasing concentrations of urate. The technical mode of procedure was as previously stated. Table II shows the values in millimoles for potassium, ammonium, lithium, sodium, rubidium, and caesium chlorides.

TABLE II  
EFFECT OF ELECTROLYTES ON GELATION OF METHYLAMINE URATE

Uric acid, %	KCl	NH <sub>4</sub> Cl	RbCl	NaCl	CsCl	LiCl
1.2	3	5	10	15	60	100
1.0	4	10	20	40	150	300
0.8	9	20	40	100	300	500
0.6	25	50	100	200	?	700
0.4	100	150	200	300	?	1250
0.3	150	200	—	—	?	—
0.2	—	—	—	—	?	—

Marked differences are apparent in the concentration of salt required to produce the same effect. It is evident in all cases that with decreasing concentrations of urate increasing amounts of electrolyte are required to attain

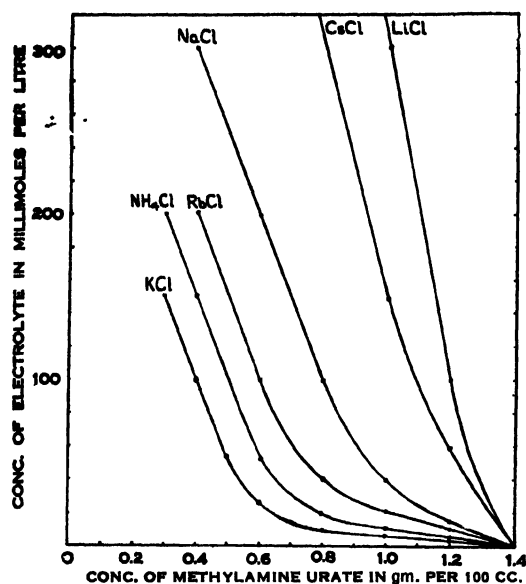


FIG. 1. The comparison of the minimal concentrations of various electrolytes required to induce gelation of solutions of methylamine urate.

the gel state. The concentration at which this power is lost also depends upon the particular electrolyte. The experiments with caesium chloride are incomplete because of the high cost of this salt, but they are sufficient to indicate the position of caesium in the group. The results are expressed graphically in Fig. 1 where the positions of the various salts are clearly shown.

Potassium chloride is taken to be the most effective salt and its action extends down to a concentration of uric acid of 0.3%. The action of ammonium chloride is very similar. "Ammonium" gels tend to form more rapidly and crystallize more rapidly than "potassium" gels. The amount of sodium chloride required for a particular concentration of urate is more difficult to determine

than that of any other salt because of rapid crystallization of the gel and the tendency of very weak gels to form. Lithium chloride is required in much the highest molar concentration to induce gelation. The order of efficacy of the cations of this group may therefore be placed as follows:  $K > NH_4 > Rb > Na > Cs > Li$ .

### Lithium Urate

The behavior of electrolytes in a supersaturated solution of lithium urate was next studied to determine whether the observed effects were applicable to other urate solutions. Lithium urate provided a gel which was much more stable than those of methylamine, forming spontaneously at 0° C. at a concentration of 2–5% and at a pH of 6–12. The procedure was slightly modified from the preceding one.

A definite weight of uric acid was suspended in water at 80–90° C., dissolved with 1 *N* lithium hydroxide, the pH adjusted to 7, the solution made up to volume and filtered if necessary. Five cubic centimetres of this solution was added to 5 cc. of electrolyte previously warmed to 50° C., the test tube was shaken and placed immediately in an ice bath. The original solutions were made double the concentration required in the experiment.

In this series the chlorides only of the alkali metals were used. The observations were extended to include the behavior of gels in the presence

of varying amounts of electrolyte. Below the minimum required for gelation the solution remained perfectly clear with or without an increase in viscosity until the time that crystallization began. Above the maximum an amorphous or crystalline deposit formed before gelation could take place.

In Table III are recorded in millimoles per litre the concentrations observed at definite levels of lithium urate.

TABLE III  
EFFECT OF ELECTROLYTES ON GELATION OF LITHIUM URATE

Uric acid, %	KCl		NH <sub>4</sub> Cl		NaCl		LiCl	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
2.0	0	1000	0	100	0	500	0	2400
1.8	0.3	1000	0.3	100	10		10	
1.0	10	1000	10	100	150	1000	150	4250
0.5	80	1500	80	200	1500	2000	400	4250
0.3	200		200		—		1250	
0.1	1500	2700	—		—		—	

NOTE:—Concentrations of electrolytes expressed in millimoles per litre.

Considering the minimal values it is evident that, as in the case of methylamine urate, potassium chloride is the most effective agent promoting gelation in a concentration of uric acid as low as 0.1%. This is, to the authors' knowledge, comparable with the lowest concentration of any solute in the gel state. The results with ammonium chloride are identical except that its influence does not extend to quite as low a concentration of urate. The inverse relation of concentration of electrolyte to concentration of urate likewise holds as with methylamine urate. The order of efficacy of the cations in this series would therefore be as follows:  $K > NH_4 > Li > Na$ . The results are plotted in Fig. 2. It is observable that in comparison with Fig. 1 the slopes of the curves are somewhat sharper at the lower concentrations of urate.

Considering the maximal values it is to be noted that, with the exception of ammonium chloride, the concentrations required to promote immediate precipitation are relatively high. Expressed in terms of percentage for the action on a

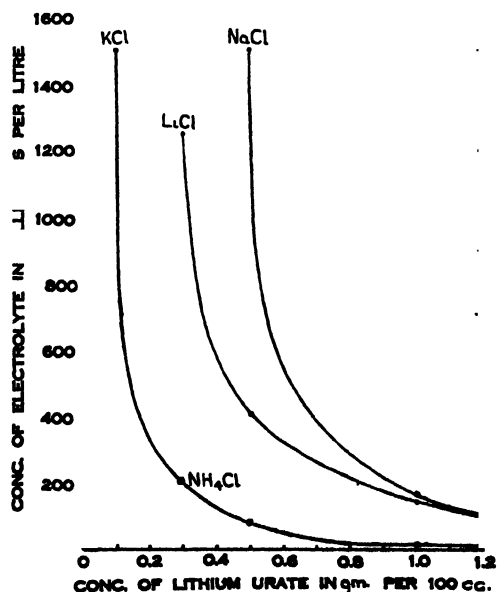


FIG. 2. The comparison of the minimal concentrations of various electrolytes required to induce gelation of solutions of lithium urate.

1% solution of lithium urate the figures are as follows (Table IV), compared with saturation values at 0° C.

TABLE IV

Concentration	NH <sub>4</sub> Cl	KCl	NaCl	LiCl
At gelation maximum	0.54	8.2	5.8	18.0
At saturation (0° C.)	29.4	27.6	35.7	67

### *Tetramethylammonium Urate*

Tetramethylammonium hydrate provides an example of a substance which forms a urate that is readily soluble. It is comparable with lithium hydroxide in its action but differs in that the viscosity of such solutions is not materially increased on cooling and no gels are formed. This urate is therefore a good example with which to test the action of electrolytes. The technique has been essentially the same as described under lithium urate. A 10% solution of the base has been used. The concentration of urate has been limited to 2% or less and the variety of electrolytes to the chlorides of the commoner alkali metals. No higher concentration than 2% of the urate is recorded because it was found that the concentration of electrolyte required was very similar. The results are recorded in Table V as minimal values in millimoles per litre of the electrolyte required to induce gelation at the particular concentration of urate.

TABLE V

EFFECT OF ELECTROLYTES ON GELATION OF TETRAMETHYLAMMONIUM URATE

Uric acid, %	KCl, 50° C.	NH <sub>4</sub> Cl, 0° C.	LiCl, 0° C.	NaCl, 50° C.
2.0	20	5	200	500
1.0	30	10	300	1250
0.5	100	20	750	2500
0.3	300	30	1250	—
0.1	1200	—	—	—

The same general result was observed as in the two previous examples. Potassium chloride was effective in the greatest dilution of urate and the results are comparable with those for lithium urate. Ammonium chloride acted in the lowest molar concentration. Its action is one of precipitation rather than gelation below 0.3% urate. It was found in the case of this salt that the temperature of the solution of electrolyte to which the supersaturated urate was added was an important factor. Thus if the initial temperature was 50° C., as previously, a voluminous amorphous precipitate resulted on adding urate, but if 0° C. a gel formed. This was also found true for lithium chloride. It will be noted that the two salts act over the same range but at very different molar concentrations. Sodium chloride is the least effective salt as with lithium urate. The order of efficacy of the cations for this series would therefore be K > NH<sub>4</sub> > Li > Na.

*Piperazine Urate*

Another example of a urate in the class of electrolyte-gelation is that of piperazine. This moderately weak base has the power of dissolving uric acid readily and forming supersaturated solutions up to about 7% at 90° C. The solubility of piperazine urate at 20° C. is about 0.5%. The technique used was the same as with lithium urate. Piperazine hydrate was added as the solid in the formation of the initial solution. The chlorides of rubidium and caesium were included in this series. In Table VI are summarized the results. The initial temperature of the electrolyte was 50° C. in all cases except ammonium chloride. In order readily to obtain gels with the latter it was found necessary to cool both electrolyte and urate solutions to about 0° C. before mixing.

TABLE VI  
EFFECT OF ELECTROLYTES ON GELATION OF PIPERAZINE URATE

Uric acid, %	KCl	RbCl	NH <sub>4</sub> Cl	CsCl	LiCl	NaCl
4 0	3	0 5	1	10	100	100
2 0	5	10	5	100	300	300
1 0	50	40	100	300	500	1000
0 5	200	100	200	500	1500	2000
0 3	400	300	300	?	—	—
0 1	1200	1200	1200	?	—	—

The cations in these experiments may be divided into three groups. The efficacies of potassium, rubidium and ammonia are about the same. Caesium holds an intermediate position. The full range of caesium chloride was not determined because of a scarcity of material, but the results obtained are sufficient to fix its position without doubt. Lithium and sodium are very similar with the former slightly more effective. The order would thus be  $K = Rb = NH_4 > Cs > Li > Na$ .

*Rate of Formation and Stability of Gels*

Throughout the experiments recorded above the intervals of time required for the formation of the gel state and the duration of the latter have been observed. The gel is broken by the crystallization of the urate which becomes microscopically or macroscopically visible. This may take place in relatively few minutes or it may require many days depending on the particular urate and electrolyte used. Without exception it has been observed that the time required for gelation varies inversely with the amount of electrolyte present in any single concentration of urate. This has been found true for all electrolytes in all preparations of the different urates. This statement is illustrated numerically in Table VII. It was observed also that the rigidity of the gel is directly proportional to the concentration of electrolyte present in any single concentration of urate up to the point of precipitation rather than gelation. These facts are of some help in interpreting the meaning of the maximum figures in Table III.

Records have also been kept of the time intervals of stability of the gels before crystallization destroyed the rigidity measured by the authors' rough method. Typical results are given in Table VII.

TABLE VII  
INFLUENCE OF ELECTROLYTES ON RATES OF GELATION AND CRYSTALLIZATION

Lithium urate, 2%			Lithium urate, 0.5%		
Conc. NaCl, millimoles	Gelation, sec.	Crystallization, hr.	Conc. KCl, millimoles	Gelation, sec.	Crystallization, min.
100	90	1	100	420	100
200	60	3	300	150	85
300	30	8	500	90	70
400	5	10	900	60	55
500	2	12	1500	15	45

Piperazine urate, 2%			Piperazine urate, 3%		
Conc. LiCl, millimoles	Gelation, min.	Crystallization, hr.	Conc. RbCl, millimoles	Gelation, min.	Crystallization, min.
300	20	> 24	2	17	45
500	9	> 24	6	8	50
750	7	> 24	10	5	60
1250	5	> 24	20	3	75

Tetramethylammonium urate, 0.3%					
Conc. NH <sub>4</sub> Cl, millimoles	Gelation, sec.	Crystallization, min.	Conc. NH <sub>4</sub> Cl, millimoles	Gelation, sec.	Crystallization, min.
100	360	20	500	10	2
300	20	8	900	2	1

It is evident that the time of crystallization varies *directly* with the concentration of the electrolytes, sodium chloride, rubidium chloride and lithium chloride, but *inversely* with potassium chloride and ammonium chloride. This has been found true for the four different preparations of urate used. Furthermore, the greater the concentration of urate at any one concentration of electrolyte the more rigid is the gel. The gels formed with lithium chloride are particularly stable, breaking up very slowly by the formation of numerous macroscopically visible nuclei in the course of days or weeks. The original gel can be melted and reformed by cooling to 0° C. if the concentration of urate is low. If the latter is high, melting is a slow procedure even at 100° C. Crystallization of these gels likewise is very slow to develop, frequently requiring many weeks. For this reason it is not practicable to include in Table VII times of crystallization for this electrolyte.

*Effect of Diamines*

The action of electrolytes in causing gelation of solutions of urates is probably one of coagulation of colloidal particles. If conditions are favorable a gel results, if not, a precipitate. The explanation of this coagulation is possible in two ways. The phenomenon may be electrokinetic in nature and as such it should conform to the facts of coagulation of typical sols and the Schulze-Linder-Picton law pertaining to valence. The curves illustrated in Figs. 1 and 2 are very similar to those obtained by Burton and Bishop (1) for the coagulation of sols of gum mastic and arsenious sulphide by univalent ions.

It has been possible in the experiments recorded above to study the efficacy in promoting gelation of cations of univalent metallic salts only. Polyvalent metallic ions have in all cases tried caused immediate precipitation of the corresponding very insoluble urate. In order to determine the effect of divalent ions it was therefore necessary to use the chlorides of soluble diamines. At a concentration of 1.8% of lithium urate, or just below the normal gelation minimum, three were effective in causing gelation as indicated below. Quinine hydrochloride was without effect. The results with a univalent amine, aniline chloride, and the chlorides of the alkali metals are also listed in Table VIII for comparative purposes. The numerical values express concentration in millimoles per litre.

This influence of the diamines is limited to a narrow range of concentration of the lithium urate.

It is thus apparent that although the diamines are effective they are no more

so than univalent ions. The results do not suggest a simple application of the Schulze-Linder-Picton law to the formation of gels. The influence of concentration of the colloid must however be taken into account as pointed out by Burton and Bishop (1). It should further be emphasized that the inverse relation of concentration of urate to concentration of electrolyte is at variance with the usual direct relation as exhibited by similar sols such as stannic hydroxide, manganese dioxide, aluminium hydroxide, vanadium pentoxide and thorium hydroxide (2). There are however exceptions to the latter rule.

*Effect of Non-electrolytes*

The phenomenon is also explicable on the basis of a lyotropic effect and it should be expected both to find non-electrolytes active in promoting gelation and to observe the lyotropic series in the efficacy of the cations studied. A summary of all the results pertaining to this point is given below:—

Methylamine series,  $K > NH_4 > Rb > Na > Cs > Li$ ; piperazine series,  $K = NH_4 = Rb > Cs > Li > Na$ ; lithium series,  $K > NH_4 > Li > Na$ ; tetramethylammonium series,  $K > NH_4 > Li > Na$ ; lyotropic series,  $Li > NH_4 > Na > K > Rb > Cs$ .

TABLE VIII

Phenylenediamine	0.5	NaCl	10
Benzidine	1	LiCl	10
Ethylenediamine	2	KCl	0.3
Aniline	10	NH <sub>4</sub> Cl	0.3



With the exception of the position of sodium in the methylamine urate series the efficacy of the ions is essentially the same in all types of urate employed,—qualitatively but not quantitatively. The order bears no resemblance to that of the lyotropic series or hydration capacity of the ions.

A further method of testing the nature of the phenomenon in supplement to those described is the effect of non-electrolytes upon gelation. Schade and Boden (9) have observed that the presence of alcohol in 50% concentration will aid the gelation of supersaturated sodium urate. Keeser and Zocher (4) have found that the addition of non-electrolytes such as urea, glucose, sucrose, glycerol and ethyl alcohol decreased the rigidity of the gels with increasing concentration and retarded the crystallization generally. Urea however was found to accelerate crystallization. The type of influence on the rigidity of the gels is the opposite of the writers' observations for electrolytes.

As a means of testing whether non-electrolytes possessed the power of causing gelation, alcohol was selected. Its effect was tested in the same way as previously with solutions of electrolytes. Double the concentration of the urate desired was added to various concentrations of ethyl alcohol at 50° C. and the mixture cooled immediately in an ice bath. The concentration of urate employed has been on the limits at which gels would form spontaneously. The effect of alcohol on the gelation of 1.7% lithium urate is shown in Table IX.

TABLE IX  
EFFECT OF ALCOHOL ON GELATION OF LITHIUM URATE

Concentration of alcohol, %	Result
10	No change in 24 hr.
20	Gelatinous ppt. becoming weak gel
30	Gelatinous ppt. becoming weak gel
40	Firm gel in 2 hr.
50	Firm gel in 1 5 hr.
60	Firm gel in 1 hr.
70	Crystalline ppt. immediately

It will be seen that alcohol in this experiment acted in a similar way to solutions of electrolytes. In low concentration the effect was not apparent; in intermediate concentration gels were formed. The rigidity and speed of formation increased with increasing amounts of the active agent; in high concentration immediate precipitation of the urate occurred.

The phenomenon of gelation in alcohol is interesting. An opalescence first appears which soon gives place to a turbidity or micro-coacervation which in turn becomes a gelatinous precipitate or macro-coacervation. The latter passes into a firm clear gel which in turn breaks down slowly depositing crystals. The less rigid gels in the lower concentrations of alcohol exhibit the phenomenon of thixotropy. The writers have also observed this phenomenon in gels of piperazine urate formed with potassium chloride. These are the only instances amongst urate gels where they have observed this effect.

As applied to lower concentrations of lithium urate the method employed yielded gelatinous, then amorphous, precipitates rather than gels. With 2% methylamine urate the results were similar to those in Table IX. With the urates of piperazine and tetramethylammonium hydrate only precipitates

were formed. In general it may be stated that those urates which form gels spontaneously will do so in the presence of alcohol but that alcohol *per se* has little or no power to promote gelation. Other non-electrolytes which were tried have been without any influence on the phenomenon of gelation. Thus urea, glucose, glycol, and glycerol were without any effect as were also the colloids, gelatin, ovalbumin and gum arabic. In high concentration some tend to cause a precipitation of the urate. Their influence on the stability of gels was not studied.

### *Sol-gel-sol Transformation*

In many experiments the writers observed the appearance of a slight opalescence in their preparations on cooling in the ice bath prior to the gelation of the solution. This phenomenon is usual in concentrated solutions of urates to which no electrolyte has been added, but not in such dilute solutions as have been employed in these experiments with electrolytes. Not only does a slight opalescence appear but this is of a graded character with increasing concentration of electrolyte. Such sols pass into gels as described above. In those experiments in which ammonium chloride has been used as electrolyte the gels break up in the course of a few minutes with the formation of brilliantly opalescent sols. A slight crystalline precipitate is deposited in the course of days, weeks or months, and the opalescence of these solutions slowly disappears by sedimentation in the course of many months. Examination of these opalescent solutions reveals them microscopically empty but ultramicroscopically brilliant with ultramicros. Such a result is strongly suggestive of the observations which have been made on agar sols under the influence of alcohol whereby they pass from an emulsoid to a suspensoid type of colloid (5, 6).

### **Discussion**

The main purpose of this investigation was achieved in the demonstration that dilute solutions of urates would gel in the presence of moderate concentrations of such common electrolytes as the chlorides of sodium and potassium. The lowest concentration of uric acid which would gel according to the writers' standard was 0.1%, but it must be emphasized that solutions of considerable viscosity exist below this concentration. It has been shown elsewhere that the blood and urine of the bird, if not of the mammal, may attain this level of uric acid concentration. The urine of the bird is normally considerably above it. The concentration of electrolyte required to induce a colloidal condition therefore becomes of importance. It may legitimately be questioned at this time whether the conditions studied have any direct application to mammalian tissues. They must have to the physical conditions of urate which exist in birds and reptiles.

A further point of interest lies in the similarity of behavior of urate solutions and the hydrous oxides such as ferrous oxide, vanadium pentoxide, alumina, silica, molybdenum pentoxide, ceria, chromic oxide in their response

to electrolytes. It is natural to ask (a) the nature of the mechanism whereby urate gels are formed, and (b) whether the present experiments have anything new to offer to the general conceptions of gel structure.

In attempting to answer the first question it is necessary to balance the evidence submitted in the experimental section. The action of electrolytes may be interpreted in two ways. It is possible to consider the state of gelation as intermediate between a supersaturated solution containing particles in both molecular and colloidal dispersions and an amorphous or crystalline precipitate—and therefore a stage in coagulation. At a given concentration of urate, it is possible by gradually increasing the amount of electrolyte to pass from a clear stable supersaturated solution which slowly deposits crystals through increasingly viscous sols and increasingly rigid gels to instantaneous amorphous precipitation. This evidence in itself the writers consider as support for the explanation as advanced by Usher (10), that a gel is a transitional stage in the formation of the precipitate. It may be considered as electrokinetic in nature as was pointed out above. The general relation of concentration of urate to concentration of electrolyte required to form a gel conforms to the behavior of electrolytes in the precipitation of such sols as those of copper, arsenious sulphide and gum mastic (1). Thus for univalent ions the concentration of ion necessary to produce a gel increases with decreasing concentration of urate very rapidly at low concentrations of the colloid. As against such a simple explanation of the phenomenon there is the anomalous behavior of the divalent ions of the diamines. These are quite effective but no more so than certain univalent ones and act over a more limited range of urate concentration.

The meaning of the maximal values recorded in Table III becomes clearer if the above explanation of gelation is accepted. A point is reached in the concentration of electrolyte beyond which the coagulation of colloidal particles is so rapid and uneven that amorphous masses result rather than regular and slower precipitation as a network with enclosed solvent or solution. That such a network exists in the gel structure is well illustrated from work with preparations of urates, where all stages of structure may be observed from viscous solutions which are almost gels and fragment on disturbance to coarsely amorphous precipitates formed throughout the liquid, from which solution may be caused to be liberated by mechanical agitation.

The behavior of the gels from the standpoint of a hydrophylic colloid may also be considered. In such a concept it would be the lyotropic power of the cation or electrolyte which would bring about the coagulation of partially dehydrated particles. As has been pointed out above the order of cations in these studies does not coincide with the lyotropic series. On this fact alone it would seem difficult to defend this explanation of urate gelation without qualification. There is further evidence on this point from the present experiments with alcohol. As shown in Table IX alcohol can act in a similar manner to the electrolytes but to a much more limited degree. It must also be remembered that we are dealing with a colloidal electrolyte itself. Furthermore alcohol has been found to act in only one of the four

urate preparations. Its behavior is not comparable for example with its action on agar sols in which the viscosity is markedly decreased with increasing alcohol strength and the preparation takes on the behavior of a suspensoid (5, 6). The action is more easily understood as comparable to the behavior of cerium oxide with alcohol (7) in which the alcohol forms alcogels and does not act primarily as a dehydrating agent.

In attempting to arrive at a picture of the gel mechanism for urates the writers would call attention to the statement of Kruyt and van der Made (7) that "only hydrated sol particles are able to cause gelation phenomena . . . that it is due to the clotting together of hydrated particles with water imprisoned between them, and that this clotting together has the character of a coagulation. It is thus a coagulation of hydrated particles. For this coagulation either the addition of a discharging electrolyte or the removal of the peptising electrolyte is required". From the above discussion it would appear that we are dealing with a case intermediate between suspensoid and emulsoid in which the character of the suspensoid is predominant, and suggestive of the behavior of the gels of alumina, molybdenum pentoxide and vanadium pentoxide; but that there are two factors of stability—hydration and ionic adsorption. For gel formation the removal of some if not all of the adsorbed ions is required.

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## CHEMICAL STUDIES ON APPALACHIAN UPLAND PODSOL SOILS

### I. CONDITIONS GOVERNING BASE-EXCHANGE RELATIONS<sup>1</sup>

BY G. T. SHAW<sup>2</sup> AND R. R. MCKIBBIN<sup>3</sup>

#### Abstract

The base-exchange properties and the constituent materials of the base-exchange complexes of Quebec Appalachian upland podsol soils have been studied. It has been shown that the "availability" of the acid semihumified organic matter has played the most important part in bringing about existing conditions in these soils. Organic matter is dominating the processes through which these podsol soils pass in their progress from a slightly leached to a severely leached condition. The inorganic base-exchange complexes are superseded by organic complexes. The more "available" iron and aluminium present in these soils the less is their base-exchange capacity. The inorganic base-exchange complexes are unstable under the strongly acidic soil conditions. The restoration of fertility to these soils must be approached through modification of their organic-matter conditions.

Investigational work (8) and Table I) has demonstrated the high degree of "availability" of a certain fraction of the organic matter and of the sesquioxides of iron and aluminium of podsol soils of the type described in this paper. Alkali treatment in small quantities effects instant separation of these fractions. The extreme ease with which some of the iron and aluminium can be removed may be further shown by leaching with  $N/2$  acetic acid (Table II). This treatment extracts surprisingly large amounts of "soluble" or "available" sesquioxides. These experiments indicate the availability of these substances, suggesting the probability that in part they exist as a coating over the soil particles and so impress some characteristics on the soil as a whole. These conditions must be linked up with the processes at work in the formation of a podsol soil. Here the organic matter is believed to be primarily responsible for the production of the acidic environment in the soil. The "available" emulsoidal fraction acts as a protective agent in the removal of colloidal matter and sesquioxides of iron and aluminium to the lower horizons. There is close association of the organic matter with these "available" sesquioxides and with the transformations that a podsol soil undergoes as the forces of podsolization proceed. All of these substances owe their "availability" and their influence on soil conditions to their attachment to the surface of the soil particles. This is particularly true of the organic fraction. As will be indicated later, surface phenomena are predominant in these soils. Only in this way may the acidic condition characteristic of podsol soils be readily explained. We have thus a picture of the

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TABLE I

THE EFFECT OF SMALL ALKALI TREATMENTS IN LIBERATING THE ORGANIC MATTER OF APPALACHIAN UPLAND PODSOL SOILS

Chemical added to 1 kgm. of soil plus 25% moisture in each case	pH value	Total solids per litre, in gm.	Organic matter in solution per litre, in gm.	Chemical added to 1 kgm. of soil plus 25% moisture in each case	pH value	Total solids per litre, in gm.	Organic matter in solution per litre, in gm.
1. NaOH — 1 gm.	6.2	0.67	0.43	4. Untreated	5.5	0.12	0.07
2. Na <sub>2</sub> CO <sub>3</sub> — 1 gm.	6.1	0.36	0.22	5. CaO — Equivalent to 1 gm. NaOH	6.4	0.24	0.11
3. KOH — Equivalent to 1 gm. NaOH	5.8	0.32	0.17	6. CaCO <sub>3</sub> — Equivalent to 1 gm. Na <sub>2</sub> CO <sub>3</sub>	6.4	0.12	0.06

NOTE:—Distilled water was leached through the treated soils in the percolators after four weeks. The first litre was collected and analyzed, and in this the soluble carbonates exerted a misleading effect. A second litre, collected after seven weeks, was analyzed and the results in Table I obtained. Several soils were examined under each treatment and the figures shown are typical.

TABLE II

THE SOLUBLE IRON AND ALUMINIUM LIBERATED FROM 100 GM. OF SOIL (CALCULATED ON MOISTURE-FREE BASIS) BY SUCCESSIVE LEACHINGS WITH 500 CC. OF N/2 ACETIC ACID

Soil	Fe <sub>2</sub> O <sub>3</sub>		Al <sub>2</sub> O <sub>3</sub>	
	Gm.	P.p.m.	Gm.	P.p.m.
1. 1st leaching	0.0007	7	0.0114	114
2nd leaching	0.0008	8	0.0122	122
2. 1st leaching	0.0033	33	0.0689	689
2nd leaching	0.0048	48	0.0521	521

NOTE:—Burgess (1) mentions that soils of pH 5-5.8 usually have about 26 p.p.m. of soluble alumina. He used a more drastic method than the writers'; he shook the soils and acid together, and let the mixture stand, while in this work immediate leaching was used.

surface action of this organic-matter fraction and a possible explanation of the consequent ease of its liberation by mild alkali treatment.

The technique employed in these studies was based on the method of Schollenberger (7), using neutral normal ammonium acetate as the agent for the removal of the exchangeable cations. A closed syphon system was used for the leaching operation. The individual basic cations were determined in the leachate after the replaceable hydrogen was determined by potentiometric back titration to neutrality. The total base-exchange capacity was found by determining the ammonium adsorbed in the soil after leaching.

Two cultivated podsol soils, representing the surface eight inches, were first analyzed for their different replaceable cations. Both soils were found to be deficient in the amounts of the individual basic cations Ca<sup>++</sup>, Mg<sup>++</sup>, Na<sup>+</sup> and K<sup>+</sup>. Highly fertile soils are known to possess quantities several

times the figures obtained. Podsolized soils are marked by this deficiency of exchangeable basic cations which reflects the low fertility of these soils.

The availability of the sesquioxides of iron and aluminium suggested the possibility that they might be present in some quantity in the base-exchange system, as other workers (2) have found when working with other types of soils. If they occurred to such an extent in these podsol soils, the fertility of the latter would probably be injuriously affected, as soluble iron and aluminium are considered to be deleterious to plant growth. However, they were found to be present to a very limited degree and it is doubtful if these traces of iron and aluminium sesquioxides really did exist in the exchangeable form. Undoubtedly they were released because of their association with the surface of the soil particles and were washed out by the leaching agent as it percolated through the soil. In these small traces they would not be able to affect soil fertility harmfully (see Table III). It is significant that the soil with more soluble iron and aluminium present has the lesser base-exchange capacity.

Another noticeable feature was the high amount of exchangeable hydrogen. In the two soils reported on, the hydrogen ions occupied 66 and 69% respectively of the total base-exchange capacity. This is termed "percentage unsaturation". The base-exchange system is quite obviously acting as a reservoir for hydrogen ions instead of being able to supply that reserve of basic cations which is so essential for fertility. A soil that has a large amount of exchangeable hydrogen soon loses a large part of its inorganic exchange complexes through disintegration into the constituent oxides of iron, aluminium and silica. In this way these constituents are converted into an available or free form and are characteristic of podsolized soil conditions. It is also poorly buffered to resist further increases in acidity through the action of additional sour organic-matter accumulation.

The picture drawn from these preliminary analyses reflects degraded soil conditions.

TABLE III

BASE EXCHANGE DATA FOR SURFACE CULTIVATED APPALACHIAN UPLAND PODSOL SOILS

Expressed as milli-equivalents per 100 gm. soil

Soil	Total capacity	Ca	Mg	Na	K	Al	Fe	H	% Base unsaturation	pH of air-dried soil	Total bases, m.e.
1	20.74	2.81	1.68	0.29	0.38	0.025	0.06	13.75	66	4.97	5.16
2	14.86	1.57	0.36	0.23	0.72	0.05	0.05	10.20	69	4.89	2.88

The analysis of the base-exchange relations of the subsoils, representing the soil lying between 8 and 18 in. in depth, again only emphasizes the low fertility from the base exchange standpoint. The "percentage unsaturation" was about identical with the corresponding surface soil layer. This indicates the

highly buffered state of the base-exchange system — the fact that it retains its acidity even in the subsoil although there is a decrease in the amounts of organic matter and of replaceable hydrogen ions in the subsoil as compared to the surface layer (see Table IV).

In order to be able to draw further conclusions about base-exchange relations, it was necessary to base them on determinations on a number of representative upland podsol soils. Eight surface soils, sampled from the surface eight inches of virgin soil, and their corresponding subsoils, sampled from the soil between 16 and 24 in. in depth, were analyzed for replaceable  $H^+$ ,  $Ca^{++}$ ,  $Mg^{++}$  and total capacity. Sodium and potassium were not determined as they are generally present in very limited quantities. The procedure for these determinations was identical with that described previously.

Table V shows the results of these analyses. It may be seen that there is a large variation in the total capacities of the different soil samples. The basic cations,  $Ca^{++}$  and  $Mg^{++}$ , show similar wide variations. In most cases the hydrogen ions constitute a strikingly large proportion of the total replaceable ions as shown by the figures for the "percentage unsaturation" (given by dividing the replaceable hydrogen by the sum of the replaceable  $Ca^{++}$ ,  $Mg^{++}$  and  $H^+$ , assuming that the sodium and potassium are present in unappreciable amounts so that they could be neglected).

TABLE V  
REPLACEABLE CATIONS\*

Soil no.	Surface soil						Subsoil					
	Ca	Mg	H	Sum	% Unsaturation	Measured capacity for base exchange	Ca	Mg	H	Sum	% Unsaturation	Measured capacity for base exchange
55	10.9	1.9	19.6	32.4	61	25.0	1.5	0.8	17.2	19.5	88	14.1
4	12.0	5.0	25.0	42.0	60	36.1	2.5	1.4	6.3	10.2	62	6.1
25	6.4	1.0	22.4	29.8	75	34.5	0.7	0.5	9.4	10.6	89	8.4
21	2.5	0.8	17.8	21.1	84	19.6	0.8	0.4	15.2	16.4	93	11.0
52	4.0	1.1	30.5	35.6	86	39.3	0.6	0.5	11.4	12.5	91	9.8
26	5.7	2.2	14.1	22.0	64	17.9	2.0	2.2	6.3	10.5	60	6.4
61	3.3	0.7	16.6	20.6	81	27.9	1.3	0.4	7.2	8.9	81	7.0
27	7.7	0.8	8.0	16.5	49	13.4	3.8	0.6	4.6	9.0	51	6.1

\*Expressed as milli-equivalents per 100 gm. of moisture-free soil.

TABLE IV  
BASE-EXCHANGE DATA ON SUBSOILS OF CULTIVATED APPALACHIAN UPLAND PODSOL SOILS\*

Soil	Ca	H	Total base-exchange capacity	% Unsaturation
1	2.73	9.15	15.27	60
2	2.56	3.91	6.24	63

\*Expressed as milli-equivalents per 100 gm. of soil.



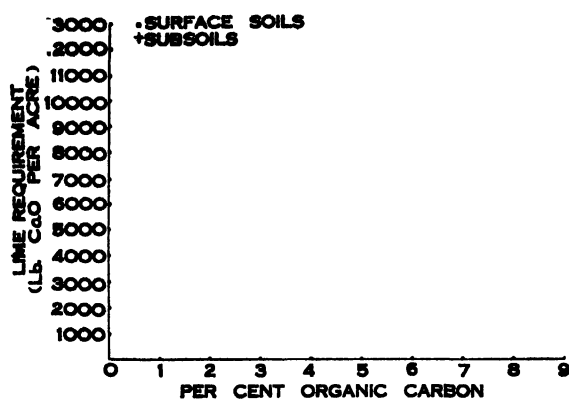


FIG. 1. Relation between lime requirement and percentage organic carbon of soils and subsoils of virgin Appalachian upland podsol soils.

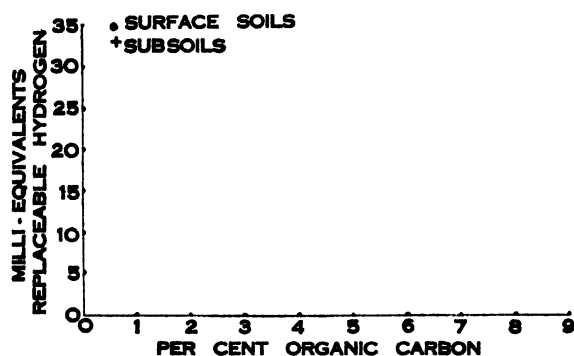


FIG. 2. Relation between the replaceable hydrogen and the percentage organic carbon of surface soils and subsoils of virgin Appalachian upland podsol soils.

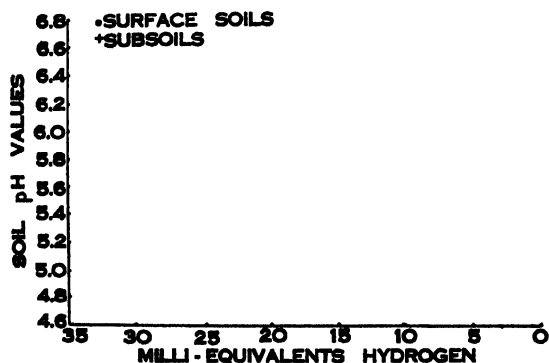


FIG. 3. Relation between soil pH values and the replaceable hydrogen of surface soils and subsoils of virgin Appalachian upland podsol soils.

It is recognized that the lime requirement is a measure of the "unsaturation" of a soil. This "unsaturation" is directly related to the acid conditions existing in the soil and may be said to be caused by the accumulation of sour semi-decomposed organic matter. The predominating influence of organic matter in an Appalachian upland podsol soil may be established by showing its relation to the lime requirement. By plotting the carbon content of each of 25 analyzed samples of virgin upland podsol soils against their corresponding lime requirement values, good agreement is obtained for the direct variation of the lime requirement with the percentage of carbon. This relation holds for both surface soils and subsoils (4) and Fig. 1). The correlation is very good and holds for all virgin soils of this type taken from this area and analyzed (5, 6).

By plotting the replaceable hydrogen of the surface soils and subsoils against their percentage of organic carbon a direct relation is indicated (Fig. 2); the replaceable hydrogen increases directly as the organic matter increases. From the evidence presented in Figs. 1 and 2 it may be stated that the soil acidity is closely associated with the organic matter and probably exists in the organic exchange complexes<sup>2</sup> to a great degree.

There is also a coincidence of degree of leaching with these factors, as measured by the thickness of the leached soil layer, and as given in Table VI. This is also indicated by plotting the soil pH values against the replaceable hydrogen, a direct line relation being obtained (see Fig. 3).

TABLE VI

ANALYTICAL DATA ON VIRGIN SOILS STUDIED IN BASE EXCHANGE WORK

Soil no.	pH value	Hygroscopic moisture, %	N, %	Organic carbon, %	C/N ratio	Thickness of A <sub>2</sub> horizon (leached layer), in.	Degree of leaching
55 Surface	4.90	8.24	0.40	8.52	21.2	1 — 3	Very heavy
Subsoil	5.13	4.00	0.13	2.68	21.2		
4 Surface	4.81	5.26	0.36	8.32	23.1	1 — 2	Very heavy
Subsoil	5.66	0.80	0.05	0.30	6.0		
25 Surface	4.88	4.86	0.07	6.12	87.9	1	Very heavy
Subsoil	5.49	2.15	0.03	1.28	45.7		
21 Surface	5.08	3.29	0.30	4.17	13.5	$\frac{1}{2}$ — 1	Heavy
Subsoil	5.31	2.01	0.07	1.48	20.2		
52 Surface	5.12	4.33	0.25	4.09	16.6	2 — 5	Very heavy
Subsoil	5.47	2.59	0.09	1.70	17.9		
26 Surface	5.59	2.53	0.12	4.00	32.6	$\frac{1}{2}$ — 1 $\frac{1}{2}$	Heavy
Subsoil	6.30	1.12	0.05	0.23	4.6		
61 Surface	5.25	2.86	0.26	3.68	13.8	$\frac{1}{2}$ — 1	Light
Subsoil	5.59	1.74	0.06	0.92	14.6		
27 Surface	6.21	2.46	0.26	2.71	10.6	0 — $\frac{1}{2}$	Very light
Subsoil	6.67	1.35	0.04	0.79	18.8		

The most significant relation is the direct variation of the total exchange capacity with organic carbon content. This is plotted in Fig. 4. As the percentage of carbon increases, the adsorption capacity of both surface soils and subsoils increases. It may be concluded that the exchange system is chiefly in the organic colloidal fraction rather than in the clay fraction. The organic complexes become more dominant as the organic matter accumulates and the inorganic complexes disintegrate.

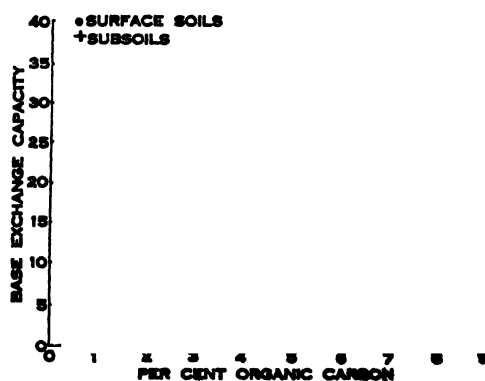


FIG. 4. Relation between the base-exchange capacity and the percentage organic carbon of surface soils and subsoils of virgin Appalachian upland podsol soils.

These deductions are also maintained by plotting the base-exchange capacity figures against the percentage hygroscopic moisture of the soils. The curve, showing that one value is a function of the other, is given in Fig. 5. The percentage of hygroscopic moisture can be taken as a relative measure of the organic colloidal fraction of these soils, the seat of the base-exchange complexes.

The phenomenon of organic base exchange has received little consideration and not much is definitely known about it. In the inorganic complexes the exchange cations are chemically united to the suspensoidal crystalline "acidoid" complex. In contrast, many organic colloids of the soil are emulsoidal. Organic matter for a given weight has a greater exchange capacity than the clay fraction. However, there is uncertainty as to how organic matter may function as a seat for replaceable ions and how they are adsorbed. It is important to note that acidity, which is not conducive to the stability of the inorganic complexes, does not act similarly on the organic

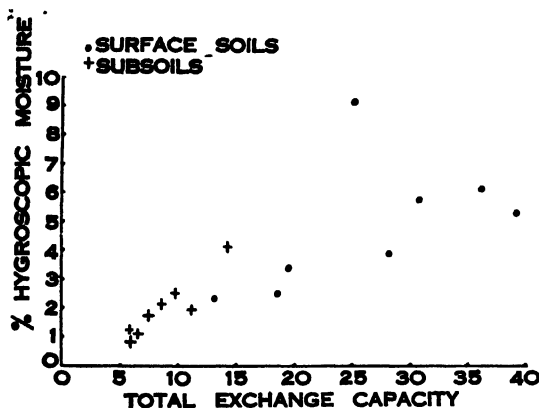


FIG. 5. Relation between the percentage hygroscopic moisture and the total exchange capacity of the surface soils and subsoils of virgin Appalachian upland podsol soils.

exchange system of these podsol soils. The exchange capacity increases as the acidity increases instead of decreasing as might be expected. Thus it may be deduced that the base-exchange system does not exist in the inorganic fraction to any great degree. Organic complexes seem to function as an adsorption system in highly acid surroundings in contrast to the instability exhibited by inorganic complexes under these conditions. Evidently the inorganic complexes once existed in these soils to a larger extent but through acidic organic matter accumulation they are disintegrating and their influence is disappearing. As podsolization progresses they are being continually supplanted by the organic complexes.

McGeorge (3) has shown that the lignin, ligno-hemicelluloses and lignocelluloses or related bodies function as the base-exchange complexes of soil organic matter. The writers' findings indicate that some relation may exist between the percentage nitrogen and the base-exchange capacity of these podsol soils (see Table VI and Fig. 6).

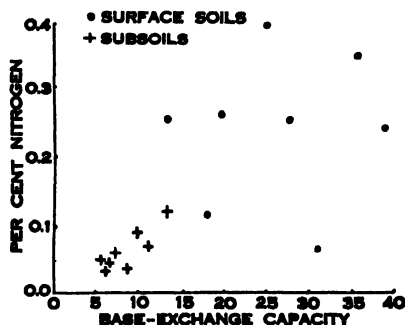


FIG. 6. Relation between the percentage nitrogen and the total replaceable capacity of the surface soils and subsoils of virgin Appalachian upland podsol soils.

No continuous relation is apparent when the amounts of replaceable calcium are plotted against the total exchange capacity. However, by plotting the replaceable calcium against the "percentage unsaturation" an interesting curve is produced. Points for individual soils are joined in a continuous line, according to the degree of leaching and the percentage of organic carbon in the soils. This is shown in Fig. 7. The upper line is for the surface soils as numbered, the lower one for the subsoils. It may be seen that the shapes of the two curves are similar and that the orders of the soils in the two curves approximately coincide. These curves illustrate that when there is either a relatively low or a relatively high amount of organic matter in an Appalachian upland podsol soil the "percentage unsaturation" is lower than it is in the case of the soils with an intermediate amount of organic matter (see Table VI). They indicate also that soils with a low "percentage unsaturation" possess a relatively high amount of replaceable calcium, although the soil concerned may have either a high or a low accumulation of organic matter. Soils with intermediate amounts of accumulated organic matter, having high "percentage unsaturation", contain relatively low amounts of replaceable calcium. The order of these soils in the curves also coincides with the degree of leaching (see Table VI), which in these soils is of course closely related to the amount of organic matter accumulation (4, 6).

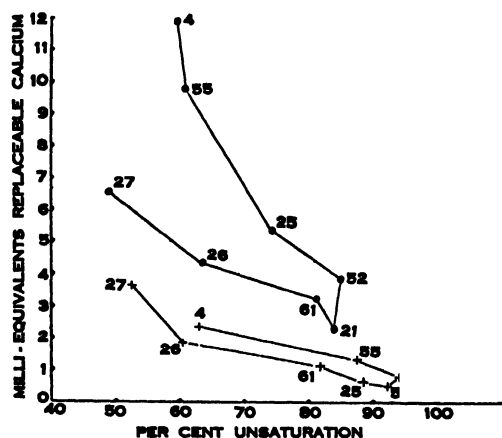


FIG. 7. The replaceable calcium is plotted against the percentage unsaturation of surface soils (upper curve) and of subsoils (lower curve) of virgin Appalachian upland podsol soils.

It may be inferred that those Appalachian upland podsol soils which have a low amount of organic matter and that are slightly podsolized have a relatively high proportion of their base-exchange systems in the inorganic clay complexes. With an increase in organic matter, podsolization increases and the soils become more acid. Thus the soils lose their lime reserves and the base-exchange complexes lose most of their basic cations, reaching a high "percentage unsaturation" as the exchangeable basic cations are replaced by the intensely active hydrogen ions.

This condition tends to produce the previously mentioned disintegration of adsorption complexes into their constituent oxides of iron, aluminium and silicon. Being freed, the sesquioxides are available for transportation to lower soil horizons through the solvent and protective action of certain fractions of the organic matter. The organic matter becomes, to a progressively greater extent, the seat of the adsorption complexes, replacing the destroyed inorganic complexes. As the new organic complexes form, the

exchange capacity increases. In time the greater portion of the adsorption system is situated in the organic fraction, with the soil becoming still more acid and podsolization more intensified due to the organic accumulations.

Unlike the inorganic complexes the organic complexes seem able to survive this condition of strong acidity for a long time. As accumulation of organic matter proceeds the organic base-exchange complexes appear to become better supplied with basic cations and have a slightly lower "percentage unsaturation". With increased base-exchange capacity in the organic fraction of the soil, basic cations may be better held and their leaching away considerably diminished. In soils Nos. 4, 55 and 25, for example, there are higher amounts of replaceable calcium, although these soils are heavily podsolized.

Some of the stages in the development of a podsolized soil may be explained by this picture. It should be noted that under the conditions in which these soils occur the lightly podsolized soils and those very heavily podsolized are more fertile than the others. The soils in an intermediate stage of organic matter accumulation are highly unsaturated in both surface and subsoil layers, which indicates that they have not sufficient exchangeable basic cations to aid in proper plant nutrition.

From the curve in Fig. 7 for the subsoils much the same conclusions may be reached. Here the conditions and influences producing alterations are not so intense as they are in the surface layer, so that the results are not so greatly divergent in soils which are existing at different degrees of podsolization. It will be noted that the curve for the subsoils is not spread out to the same extent as that representing conditions in the surface horizons. The same type of curve is given by replaceable magnesium.

In virgin podsol soils the surface horizon is the seat of the accumulated organic matter which is the cause of the processes active in podsolizing a soil. From this centre the activity associated with the effect of the organic matter commences to function and leaching occurs. Soluble electrolytes are gradually washed down and iron and aluminium are carried below. These influences remove the "available" minerals, and organic matter forms a coating over the soil mineral particles. The inorganic exchange system is supplanted by an organic one. The replaceable mineral or basic cations are largely displaced by the non-metallic hydrogen ion. In the manner described the upper horizons of a podsol soil are transformed from a mineral system to an organic one. According to this viewpoint podsolization is a demineralization process—the dominance of, and the effect of, certain fractions of organic matter on the reactivity of a soil. In the bleached horizon, the true "podsol" horizon, little colloidal material remains, either of an organic or inorganic nature.

From these data and from this discussion it may be seen that organic matter is believed to play a leading role in these podsol soils. It is the key through which their restoration to a more fertile plane must be attempted. The present state of the organic matter appears for many crops to be positively

harmful and it requires modification before the soils can be expected to become more fertile. Desirable alteration of its physical and chemical condition naturally aids in alleviation of the high lime requirement.

It is believed that any specific treatment may not be applicable to all upland podsol soils in attempting to increase their fertility. Soils only slightly podsolized and those in an advanced state of organic matter accumulation have been shown to be fairly well supplied with replaceable bases that are essential for plant growth. Such soils apparently need not be treated for their restoration in the same drastic manner as those in an intermediate podsolized condition. A modified treatment is required to suit the degree of podsolization. Soils which have attained relative "podsollic" maturity may be considered to have become dominated by the influences causing demineralization and coating of the mineral particles in the surface horizon by the organic film. In an immature podsol soil of the type discussed here transformational influences do not completely predominate and the soil retains its mineralized condition to a greater extent. Thus, it will be readily understood that the stage of development of podsolization will have an important bearing on whatever remedial applications are made to these soils.

These studies of base-exchange conditions illuminate the significant role of the acid organic matter accumulation in lowering the fertility of these Appalachian upland podsol soils.

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# THE DENSITIES AND PARACHORS OF VINYL ACETATE AND ITS LIQUID POLYMERS<sup>1</sup>

BY CHARLES GREEN<sup>2</sup>, JAMES MARSDEN<sup>3</sup>, and A. C. CUTHBERTSON<sup>4</sup>

## Abstract

The density of monomeric vinyl acetate has been determined over a range of temperature. The parachor and Ramsay and Shield's constant have been obtained for the monomer and some liquid polymers. On the assumption that a liquid polymer may be considered as a solution of a dimer in the monomer, parachors were calculated from the mixture law.

If Staudinger's formula be assumed for the dimer the calculated and observed parachors agree to within 1%.

In many cases polymerization is characterized by the repeated addition of a structural unit, and the substance when liquid passes successively through stages of increasing viscosity and eventually to the solid state.

In the course of an investigation of the polymerization of vinyl acetate some additive and constitutive property might in the authors' opinion serve as a guide to the mechanism of such a reaction in its initial stages. As Sugden's (5) parachor is both an additive and constitutive property of liquids the value of this quantity was determined during the initial stages of the polymerization of vinyl acetate. This substance was chosen because the degree to which it polymerizes is readily controlled and the available evidence points to a chain type of reaction. The density of vinyl acetate was determined over a temperature range.

## The Density of Vinyl Acetate

The vinyl acetate\* was distilled through a long spiral column to remove traces of acetaldehyde, acetic acid and copper acetate present as an inhibitor. The density measurements were carried out in a dilatometer. The values obtained are given in Table I.

TABLE I  
DENSITY MEASUREMENTS OF VINYL ACETATE

Temp., °C.	Density, $D_4^{20}$	Temp., °C.	Density, $D_4^{20}$	Temp., °C.	Density, $D_4^{20}$	Temp., °C.	Density, $D_4^{20}$
9.0	0.9459	16.65	0.9360	20.00	0.9312	25.00	0.9243
10.40	0.9441	17.00	0.9355	21.00	0.9300	25.75	0.9235
12.50	0.9413	18.40	0.9336	22.00	0.9288	30.00	0.9181
15.00	0.9381	19.00	0.9327	24.00	0.9260	31.50	0.9161

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\* Kindly supplied by the Shawinigan Chemical Co.

Within the accuracy of the results, 1 in 5000, the relation between density and temperature is linear. The equation  $D_4^t = 0.9446 - 0.001326(t-10)$  represents the above data, where  $D_4^t$  = density, and  $t$  = temperature in °C.

#### *The Parachor of Vinyl Acetate and its Liquid Polymers*

According to Sugden (5) the parachor is given by

$$P = \frac{M(y)^{\frac{1}{2}}}{D-d},$$

where  $P$  = parachor of a substance,  $y$  = surface tension,  $M$  = molecular weight,  $D$  = density of the liquid, and  $d$  = density of the vapor. Usually  $d$  is neglected so that the expression becomes  $P = \frac{M(y)^{\frac{1}{2}}}{D}$ .

#### *Surface Tension Measurements*

The surface tension was measured by the capillary rise method as approved by Richards (3). A fine capillary tube was sealed to a large tube, 4 cm. in diameter, bent in the form of a U, so that the planes were as nearly parallel as possible. The capillary was chosen from a number for its regularity in bore and because its cross section was almost a perfect circle.

#### *The Radius of the Capillary*

The radius was determined by measuring the length of a column of mercury in the capillary with a travelling microscope. A correction was made for the protruding meniscus according to Winkler (6) and also on the assumption that for small capillaries the meniscus was a hemisphere. From two determinations the radius by the latter method was 0.02555 cm., by the former 0.02552 cm. Using the value of 0.02555 cm. the surface tension of water at 25°C. was found to be 71.70 dynes per cm., in good agreement with the accepted value 71.90.

#### *Molecular Weight Determinations*

The molecular weights of the polymerized products were determined by the conventional Beckmann freezing point method, using benzene as solvent. The effect of the solvent was investigated by using nitrobenzene and comparing the values for the molecular weights obtained. This was done for the monomer. No anomalous behavior was noted. The polymers are either insoluble or difficultly soluble in polar solvents.

#### *The Densities*

The densities were measured in the same dilatometer which had been calibrated for the determination of the density of the monomeric vinyl acetate previously mentioned.

#### *The Preparation of the Samples of Vinyl Acetate*

After distillation of the vinyl acetate in air, about 15 cc. was placed in each of five test tubes which had been previously cleaned in chromic acid solution, washed with distilled water and dried in air. These tubes were sealed off, following which they were exposed to light and heat from tungsten lamps enclosed in a reflecting tin box. The temperature was about 137° C. A sample was removed every two hours.



The results for surface tension, molecular weights and densities of the six samples are given in Table II. The parachor and Ramsay and Shield's constant are shown in Table III.

TABLE II

SURFACE TENSION, MOLECULAR WEIGHTS AND DENSITIES OF SIX SAMPLES OF VINYL ACETATE

Sample	Temp., °C.	Surface tension, dynes per cm.	Density	Wt. of vinyl acetate	Wt. of benzene	Temp. drop	Mol. wt.
I	20	23.95	0.9312	0.1716	15.806	0.637	86.38 <sup>*</sup>
	25	23.16	0.9243				
	30	22.54	0.9181				
II	20	23.89	0.9548	0.1371	16.420	0.453	94.39
	25	23.48	0.9482				
	30	22.81	0.9417				
III	20	24.15	0.9603	0.1371	17.592	0.409	97.57
	25	23.53	0.9536				
	30	.....	0.9471				
IV	20	24.10	0.9638	0.1196	17.159	0.365	97.74
	25	23.55	0.9573				
	30	22.91	0.9509				
V	20	24.31	0.9709	0.1258	17.491	0.360	102.3
	25	23.70	0.9634				
	30	23.01	0.9571				
VI	20	.....	0.9716	0.1234	17.570	0.326	110.4
	25	23.61	0.9648				
	30	.....	0.9581				

TABLE III

THE PARACHOR AND RAMSAY AND SHIELD'S CONSTANT FOR SAMPLES OF VINYL ACETATE

Sample	Parachor	Ramsay and Shield's const. 20-30°C.	Sample	Parachor	Ramsay and Shield's const. 20-30°C.
I	204.5	-2.41	IV	224.8	-2.14
	205.0			225.0	
	204.5			224.9	
II	218.5	-1.88	V	234.0	-2.38
	219.2			234.4	
	219.1			234.2	
III	225.2	20-25°C.	VI	252.2	z
	225.3	-2.22			

### Theoretical

It is assumed that few if any of the molecular species present in the vinyl acetate at the stages of polymerization considered are higher than dimers. On this basis the degree of polymerization may be calculated from the following formula,

$$\alpha = \frac{M_o - M_i}{M_o(1 - 1/n)}$$

where  $\alpha$  = degree of polymerization,  $M_o$  = observed molecular weight,  $M_i$  = molecular weight of monomer = 86.05, and  $n = 2$ .

The degree of polymerization of the samples has been calculated and is shown in Table IV.

TABLE IV  
POLYMERIZATION OF VINYL ACETATE

Sample	I	II	III	IV	V	VI
Degree of polymerization	0	0.177	0.236	0.239	0.318	0.441

Assuming the presence of a dimer of molecular weight  $2 \times 86.05 = 172.1$  the mole fraction of the dimer may be calculated in each case, *i.e.*, mole fraction of the dimer in II

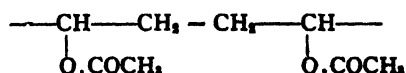
$$\frac{\frac{0.177}{172.1}}{\frac{0.177}{172.1} + \frac{0.823}{86.05}}$$

The mole fractions are given in Table V.

TABLE V  
THE MOLE FRACTIONS OF MONOMER AND DIMER IN THE VARIOUS SAMPLES

Sample	Mole fraction dimer	Mole fraction monomer	Sample	Mole fraction dimer	Mole fraction monomer
I	0.	1.000	IV	0.136	0.864
II	0.097	0.903	V	0.189	0.811
III	0.134	0.866	VI	0.283	0.717

Staudinger (4) has suggested that vinyl acetate polymerizes according to the following scheme



If it be assumed that vinyl acetate, polymerized to the states in question, is a mixture of the monomer and dimer it was interesting to find whether for a given sample, the mole fraction of the dimer  $\times$  parachor of the dimer + the mole fraction of the monomer  $\times$  parachor of the monomer = the observed parachor as determined experimentally. Taking the atomic values\*, C = 4.8, H = 17.1, O<sub>2</sub> in esters = 60; the parachor for the dimer = 363.6.

Table VI shows the observed and calculated values of the parachors of the six samples.

TABLE VI

THE OBSERVED AND CALCULATED VALUES OF THE PARACHORS OF THE SIX SAMPLES

Sample No.	Parachor		Diff., calcd.-obs.	Sample No.	Parachor		Diff., calcd.-obs.
	Obs.	Calcd.			Obs.	Calcd.	
I	204.7	205.0	0.3	IV	224.9	226.5	1.7
II	218.9	220.4	1.5	V	234.2	235.0	0.8
III	225.3	226.7	1.4	VI	252.2	249.9	-2.3

### Discussion

In the calculations referred to, the assumption has been made that the dimeric vinyl acetate is dissolved in the monomer. If  $x$  is the mole fraction of the dimer,  $P_x$  = parachor of the dimer,  $P$  = parachor of the monomer and  $P_m$  the parachor of the solution,  $P_m = P(1-x) + P_x x$ . Hammick and Andrew (1) have applied this equation to the determination of the parachors of organic liquids in polar and non-polar organic solvents. They found that the mixture law gives either the value of the parachor or a series of values from which the true value can be obtained by extrapolation.

The application of the mixture law to vinyl acetate is of course justified only in so far as the calculated and observed parachors of the liquid polymers agree. A certain difficulty arises in the measurement of the surface tension of viscous solutions. This difficulty might be overcome by dissolving the polymer in benzene and applying the mixture law to the solution obtained.

The value for the Ramsay and Shield's constant of the monomer is somewhat higher than the average value for a normal liquid. A number of acetates however give a value of 2.3, others as high as 2.6. The value of 2.4 is therefore in good agreement. Judging from the values of the constant the polymers act like associated liquids with the distinction probably that, while the phenomenon of association is reversible, polymerization is not.

\*Mumford and Phillips' (2) values gave a calculated parachor of 201.2 for the monomer allowing for a value of  $-6$  for strain constants.

Staudinger's (4) formula for vinyl acetate is based on the evidence that the decomposition product of the polymers is oxalic acid, and on the assumption that it is a chain reaction. The agreement between the calculated and observed values for the parachor confirm this formula.

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AN EXPERIMENTAL INVESTIGATION OF PROBABILITY<sup>1</sup>BY A. L. CLARK<sup>2</sup>

## Abstract

An experimental determination of probability is described in this paper. Steel balls were dropped on to a horizontal plate pierced with holes in regular array, and the number which passed through without contact with the plate was observed. The ratio of the number of free passages to the total number of balls dropped is the experimental probability, and this number was found to be in very close agreement with the probability calculated from measurement of the balls and holes. Since this calculated probability involves  $\pi$ , the experimental probability may be used to calculate  $\pi$  which was found to be  $3.143 \pm .005$ . The paper closes with a brief discussion of the application of probability to certain problems in physics.

The experiment described in the following paper was evolved as a laboratory experiment for third year honor students in physics. An experiment was sought which would combine freedom from monotony, easy discrimination between favorable and unfavorable cases and speed of operation and have the added interest of furnishing a method for deducing the value of the constant  $\pi$ .

The well-known problem of Buffon (2, p. 98) as the basis of an experiment was rejected because it lacks the first three desiderata although it contains the interesting feature of yielding the value of  $\pi$ .

It is perhaps unnecessary to say that a contact with probability through an experiment is highly desirable for students of physics and mathematics.

Steel balls are dropped vertically at random on to a horizontal steel plate which is bored with circular holes in hexagonal array and the observer records whether or not they pass through the holes without contact with the plate. The probability that a ball dropped at random will make a free passage is given by:

$$P = \frac{2\pi (R - r)^2}{d^2 \sqrt{3}} \quad (1)$$

for the hexagonal array, or would be

$$\frac{\pi (R - r)^2}{d^2}$$

for a square array.  $R$  and  $r$  are the radii of holes and balls respectively and  $d$  is the centre distance.

The hexagonal array was chosen because it gives a slightly larger  $P$  for the same distance  $d$ . It is desirable that the probability should not be small for then the interest would suffer. The distance  $d$  must not be too small compared with  $R$ , otherwise there would be a large distortion of the holes already bored when boring the adjacent holes.

<sup>1</sup> Original manuscript received June 19, 1933.

Contribution from the Department of Physics, Queen's University, Kingston, Ont., Canada. This paper was read at the May meeting of the Royal Society of Canada, 1933.

<sup>2</sup> Dean of Applied Science, Queen's University.

### Apparatus

The plate (Fig. 1) is of steel about 1 ft. square and  $\frac{1}{4}$  in. thick. It is suspended on supporting arms on a shallow frame  $1\frac{1}{2}$  ft. square. This frame is closed on the lower side by a cloth shaped like an inverted tent which serves the double purpose of catching the balls without sound and delivering them to a small cup through a hole at the vertex of the cone. The plate may be moved six inches and is treated as though it were infinite in extent.

The dropper proper, shown in Fig. 2, consists of a trough which acts as a hopper and allows the balls to run freely into the vertical dropping tube. The lower end of this tube is closed by a sliding plate pierced with a  $\frac{3}{16}$ -in. hole. This plate is placed at the end of a flat spring which is pushed forward by a plunger operated by an electromagnet. Attached also to the spring is a tongue of metal which moves in between the lowest two balls when the plate moves forward to allow a ball to drop from the tube. Thus one and only one ball at a time is allowed to fall.

The balls fall from the dropper into a shallow pan-shaped funnel in which they roll to the centre into the vertical delivery tube through which they fall on to the plate. This delivery tube with the attached pan is supported on horizontal rods which rest on a frame about three inches square. The delivery tube may be moved through a range of three inches and, no matter where the plate may be, is always over the holes in the plate.

The plate and delivery tube are moved after each ball is released and the double motion provides the randomness which motion of either alone might lack. The operator might fall into a routine with one motion only but is less likely to do so with the two. When the apparatus is used by students, a screen is placed in front of the delivery tube so that the person moving the plate does not see what the one moving the delivery tube has done. The results show that randomness, the essence of a probability experiment, has been achieved. Fig. 3 shows the assembled apparatus.

Fifty balls are placed in the hopper and released one by one. The record is made by indicating by symbols in the record book whether or not the ball touched the plate. A microphone is attached to the lower side of the plate and is in series with a single dry cell and a pair of ear-phones. This micro-

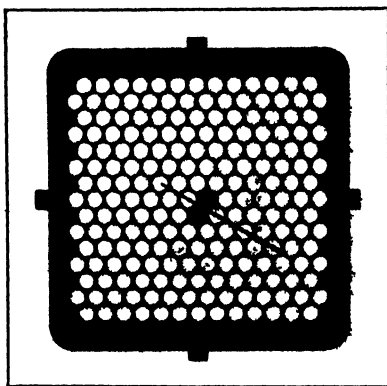


FIG 1 *The plate.*

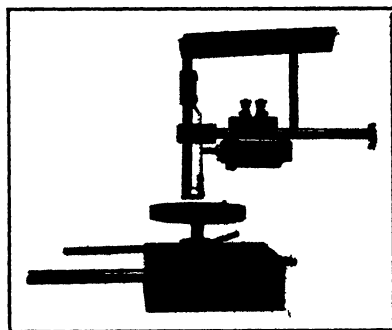


FIG. 2. *The dropper.*

phone is the carbon capsule from an ordinary Northern Electric telephone transmitter. The slightest contact of a ball with the plate gives a decided noise in the phones. While there may be a slight doubt as to just what constitutes a contact, the region of uncertainty must be very small indeed. If a sound is heard, contact is assumed; if there is no sound, a free passage is recorded.

Observations may be made and recorded with great rapidity. Even inexperienced observers can digest the problem and record 500 trials in a two-hour laboratory period. The results obtained by each pair of students are

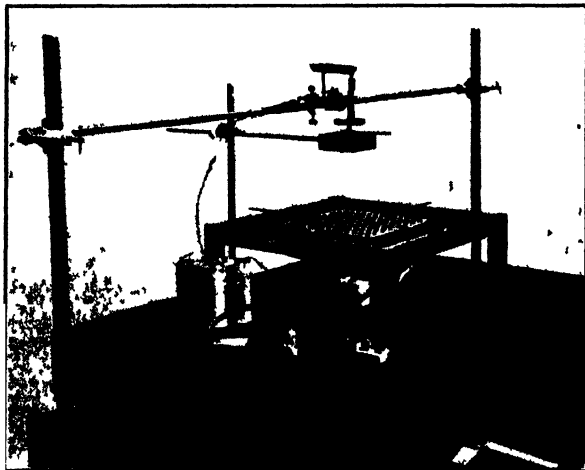


FIG. 3. *The assembled apparatus.*

recorded in a log of the experiment and the experimental values of the probability by 100's and for all observations to date are drawn day by day on a continuous graph.

The results which follow were obtained by various observers but largely by Mr. E. Harris, Curator in the Physics Department, whose interest and enthusiasm led him to carry the record to 100,000 events after the goal of 50,000 had been reached.

### Measurements and Errors

The diameters of the balls were found by measuring many random samples. The accuracy with which these balls\* are made is extraordinary. Not only do single balls vary but little but the variation of the diameters of different balls is almost nil. The diameters were measured with two micrometers, one a new metric instrument, the other an excellent shop micrometer graduated in inches. These were checked with Johannsen gauges. The diameter was found with a variation of not over 0.0001 and is taken as 0.1872 in.

The diameters of the holes and the distances between centres were found by using two steel cylinders carefully made with an almost infinitesimal taper. These fit the holes snugly and are calipered for the diameter. The diameter of the holes is 0.6572 in. For the centre distance, the cylinders are pressed into adjacent holes and a tapering steel scale pushed gently between them. This scale is five inches long and is graduated into 50 parts. Each of these parts represents  $\frac{1}{1000}$  in. so that readings may be made by estimation to 0.0001 in. The cylinders and scale are shown in position in Fig. 1.

\* The balls used are the regular Hoffman balls for bearings.

The mean centre distance is 0.7507 in. for the horizontal lines of holes, and 0.7499 in. for the inclined lines. Since operations involving  $\sqrt{3}$  are not contemplated in the construction of a milling machine it is not possible to get the two sets of distances alike. The results show also that the main screw of the milling machine is not quite exact. What was supposed to be 0.75 is 0.7507 in.

Since the two sets of centre distances vary, a better expression for the probability is

$$P = \frac{2\pi (R - r)^2}{a\sqrt{4b^2 - a^2}} \quad (2)$$

where  $a$  is the mean of the horizontal and  $b$  that of the inclined distance.

A source of error not foreseen comes from the fact that the balls do not fall quite vertically. If the plate were infinitely thin, this would not matter very much since the departure from the vertical is small. Looking along the direction of the tangent to the path where it meets the plate, one sees the upper circle of the hole as slightly elliptical, but sees also the wall of the hole. A ball moving along this direction may fall into the hole only to meet the wall. The effective area of the hole is reduced therefore by an amount depending on the amount that the path departs from the vertical.

It is possible to calculate in turn, the vertical component of the velocity, the horizontal component and the angle between the path and the normal to the plate. Then the reduction in area of the hole may be calculated for a series of distances from the centre line and a curve drawn. We have then the means of determining the effective area for any distance from the centre. A number of spotting runs of 50 each were made with carbon paper, the central point located for each with a plumb bob and a target diagram drawn. By counting the number of spots in each ring a fair estimate of the mean error may be found. A number of such tests yielded the mean factor 0.9983 which must be multiplied into the value of  $P$  calculated from the measurement. The error in this factor is less than those already discussed.

When all sources of error are taken into account the value of the probability of a free passage is

$$P = 0.3554 \pm .0005.$$

## Results

According to our notions of probability, the larger the number of events, the more closely the experimental probability should approach the theoretical value. The experimental probability in any series is the ratio of the number of free passages to the total number of cases. Hence if the total number of events is continually increased, the graph of the experimental probability should approach the theoretical value or should approximate a straight line.



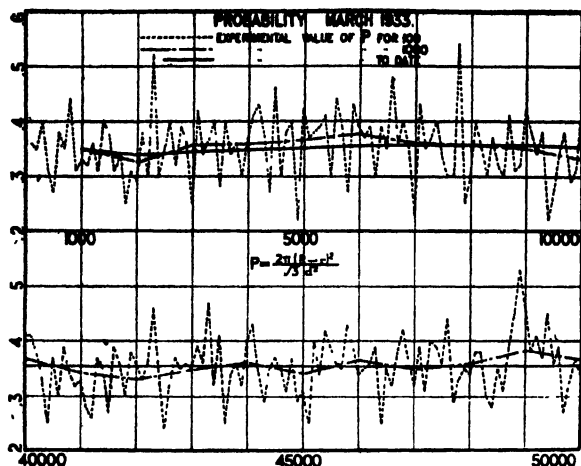


FIG. 4. The values of the experimental probability by 100's.

The diagram of Fig. 4 shows the values of the experimental probability by 100's, where the saw-shaped broken line shows the experimental values and the full line the cumulative value or the "value to date." Each point on this graph represents the value of the experimental  $P$  for all the observations taken to that point. The first and fifth tens of thousands only are given in order to show the straightening out of the graph.

Fig. 5 shows the same thing but plotted to a very much larger scale and by thousands instead of by hundreds. The graph of the value to date is carried to 100,000 and again straightens out very clearly. The terminal value cannot be far from the truth. The scale was again enlarged and the results plotted (Fig. 6) by 5000's and by 10,000's. The cumulative value calculated from the 5000 groups again shows the approach to a mean.

Since the result at the end of a series is not necessarily the best result, we may attempt to find the true mean by inserting a value between those that seem too high and those that seem too low. On the low-scale diagram this would show nothing, but on the large-scale diagram the method is fruitful. By this method we arrive at the value 0.35556 (see Fig. 6).

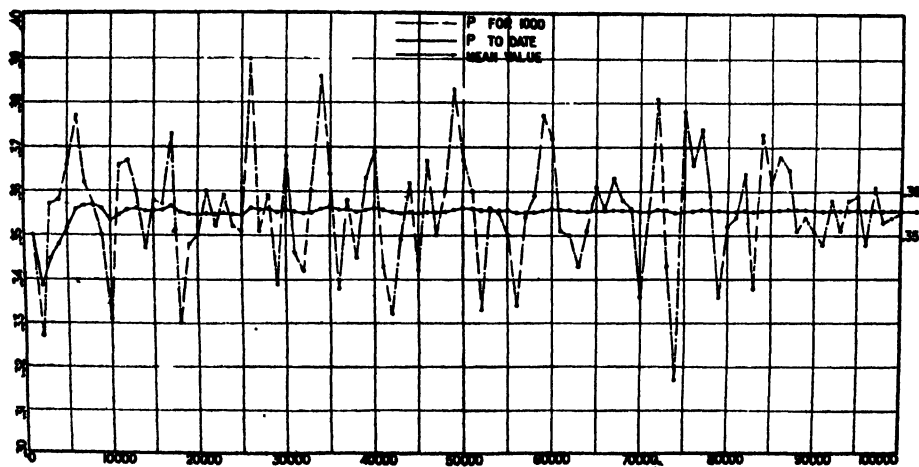


FIG. 5. The values of the experimental probability by 1000's.

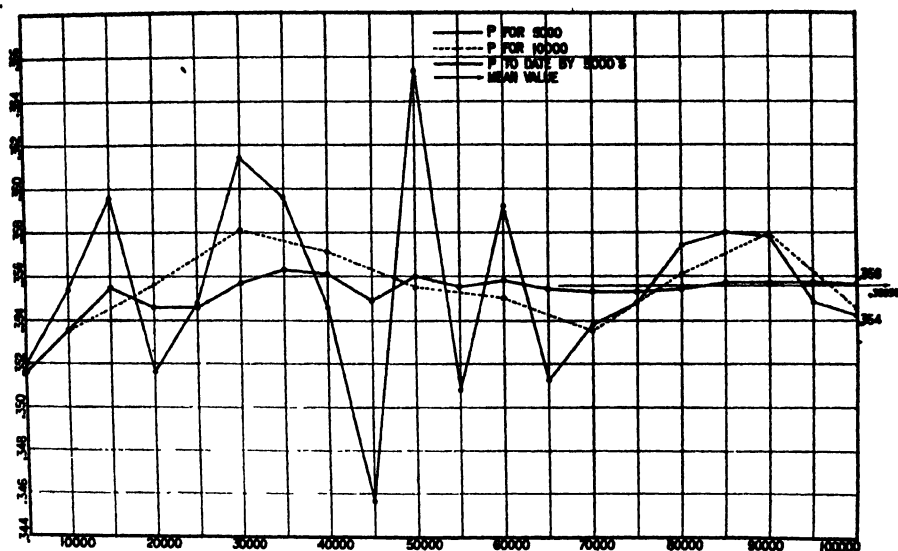


FIG. 6. The values of the experimental probability by 5000's and 10000's.

The terminal value is 0.3558. The value 0.3556 may be taken as a very close approximation indeed to the true value and is certainly more accurate than the calculated value.

The agreement between calculated and observed values of  $P$ , while gratifying is a little misleading. It is closer than the accuracy of measurement of the radii and centre distances might be expected to give and shows perhaps that the errors in these measurements partly neutralize each other.

If the experimental values of  $P$  by 100's are plotted against the number of times that each occurs we have a very good picture of the ordinary probability curve (Fig. 7). This curve also shows the fluctuations about the mean, taken here as 0.36. The small fluctuations appear often, the large seldom.

These fluctuations are very interesting. If we take extreme values of the number of free passages for groups of series of different lengths the graph of the fluctuations (Fig. 8) is of startling smoothness.

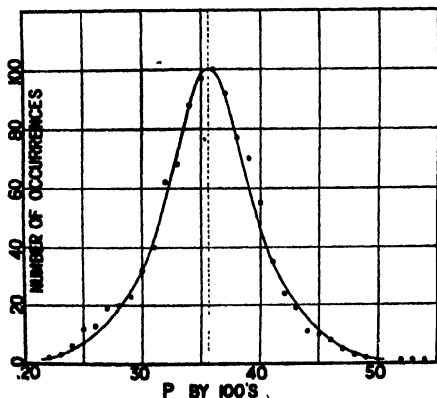


FIG. 7. Distribution of values of  $P$ .

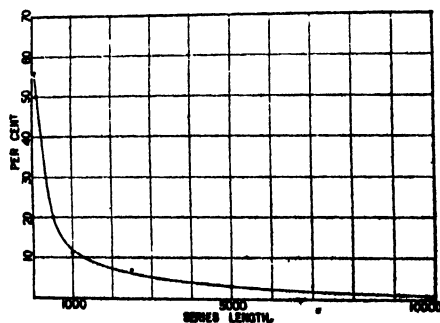


FIG. 8. Per cent maximum fluctuations.

Table I shows the values of  $P$  by 5000's and by 10,000's, and of course the fluctuations.

TABLE I  
VALUES OF  $P$  BY 5000's AND 10000's

	By 5000's	By 10000's		By 5000's	By 10000's		By 5000's		By 5000's
1	0.3516	0.3535	6	0.3614	0.3550	11	0.3508	16	0.3574
2	0.3554	0.3556	7	0.3596	0.3525	12	0.3592	17	0.3580
3	0.3596	0.3581	8	0.3546	0.3561	13	0.3512	18	0.3578
4	0.3516	0.3571	9	0.3456	0.3579	14	0.3538	19	0.3548
5	0.3548	0.3555	10	0.3654	0.3545	15	0.3548	20	0.3542

### Sequences

Finally and perhaps what is most important is the table of sequences (Table II). If  $P$  is the probability of a favorable event,  $P^2$  is the probability that there will be two such events in succession,  $P^3$  that there will be three,

TABLE II  
SEQUENCES IN 100,000 EVENTS.  $P = 0.3556$

	Runs calculated	Runs observed		Runs calculated	Runs observed
1's	35,560	35,558	8's	26	33
2's	12,676	12,259	9's	9	17
3's	4497	4655	10's	3	9
4's	1599	1489	11's	1	5
5's	569	572	12's	.4	3
6's	202	191	13's	.1	1
7's	72	74			

etc. Then if  $n$  is the total number of events,  $k$  the number in a sequence, the number of such sequences is

$$N = P^k(n - k + 1).$$

If  $n$  is very large compared with  $k$  this becomes

$$N = P^k n.$$

The table shows the calculated and observed values of  $N$  for 100,000 trials. Attention is called to the occurrence of sequences of 12 and 13 which appeared "out of due season." These are all the more important and interesting because of their early appearance and will be referred to later. Since a 13 counts as two 12's, three 11's, etc., the 12 and 13 observed make the numbers of 8's, 9's and 10's a little larger than they should be. The effect on shorter sequences is unimportant.

### The Determination of $\pi$

Obviously if 0.3554, the calculated value of  $P$ , is substituted in (2) corrected, the value of  $\pi$  (3.1416) used in the calculation comes out. If 0.35556, the observed value of  $P$ , is used the value of  $\pi$  is slightly larger than the correct value, *viz.*, 3.143. This is the best value obtainable if the value of  $P$  is determined properly, *i.e.*, the result of a predetermined random series. If however, a value of  $P$  were to be selected from the observations a closer value of  $\pi$  might be obtained, indeed a very high degree of precision might result. The experiment however cannot be expected to give a value closer than 0.1% and any such selection is indefensible.

The celebrated problem of Buffon has been used by a number of observers to determine  $\pi$ . La Place seems to have been the first to suggest such a use. The problem may be stated thus. If a straight rod or needle of length  $c$  is cast at random on to a horizontal plane surface ruled with a series of straight parallel lines at a distance  $a$  apart, where  $c > a$ , what is the probability that the rod lies across one of the lines? As shown by Buffon the value is

$$P = \frac{2c}{\pi a} .$$

Wolf (2, p. 169) in 1850 using 5000 casts found the value of  $\pi = 3.596$ . Smith in 1855 with 3204 casts obtained 3.1553, Fox in 1894 with 1120 casts, 3.1419 and Lazzarini in 1901 with 3408 casts arrived at the astounding value 3.1415929.

All except Wolf seem to have selected the most favorable place to end the experiment. No such accuracy as indicated would be possible from a random series. The results therefore are not important.

The experiment of Buffon has a certain advantage over the experiment described in this paper. Only two measurements,  $a$  and  $c$ , are necessary and these measurements enter the calculation in the first power only, so that the errors are not magnified. In the ball experiment there are three measurements and they enter the problem in the second power so that the individual errors are doubled.

On the other hand there must be a number of cases where it is difficult to tell whether the end of the needle lies on a line or not. In the ball experiment, contact with the plate is easily determined if the microphone is used. While even then there must be a very small indefinite region where there is a little doubt about contact there is no doubt whatever about the response of the microphone. The noise, if any, is loud and unmistakable and there is no gradation of the sound.

It is probably as easy to caliper smooth cylindrical or spherical surfaces as to measure distances between such lines as would be used in an experiment. From the point of view of interest, the ball experiment is superior to that based on the problem of Buffon. There is a stimulation of interest in the waiting for the click and in the movement of the plate. The observations are made very rapidly and the monotony of the observation is not excessive. The balls are not returned to the hopper until fifty observations have been made.

### General Remarks

While the results of experiments in probability prove very little, the conformity with theory exhibited in this experiment and the close approximation of the experimental to the calculated results are significant. And while one should not base arguments on these results alone, they suggest applications and lead naturally to the discussion of important points. Some of these points will now be taken up.

In the writings of certain authors, there is an implication that the extremely improbable in a series of events will happen if the series is sufficiently extended. Chwolson (1, p. 453), gives an example of this kind of statement. There, in what is one of the best treatments of the second law of thermodynamics the writer has seen, the idea of waiting for an improbable event which will happen if the time is long enough, is emphasized. The more improbable the event, the longer one must wait. As a matter of fact, the improbable may occur early in the course of events, or it may not occur at all. There is no reason why the improbable should be relegated to the distant part of a series of events or why it must certainly occur. One place in the series is as favorable as another.

The suggestion that if an improbable phenomenon has at any time not yet occurred, it is only necessary to wait for it to come later, is a trifle naive, and the explanation of its failure to occur may be given at a much later time with exactly the same assurance.

There is another kind of implication in writings of quite another sort, the so-called popular treatment of modern scientific discovery seen so often in the newspapers of our day. Such statements as "a kettle of water over a fire boils instead of freezes simply because boiling is more probable than freezing." One may infer that the improbable freezing might happen somewhere at some time. No one has ever heard of such a phenomenon nor does any one for a moment expect that it will ever happen. Such statements are misleading and the writer believes them untrue.

If we turn for a moment to the experimental work described in this paper, some light may appear. Consider first the fluctuations. If the experimental probability is calculated from groups of 50 observations each, the values lie between 8 and 28, *i.e.*, there are fluctuations about the mean, as large as 10, or the percentage fluctuation is 56. For groups of 100 the percentage is 50, for 500 it is 20, for 1,000 it is 11, etc. (Fig. 8).

As would be expected, the percentage or relative fluctuation falls off rapidly as the series length increases. For groups of 100,000 each we may predict a low value safely, for 1,000,000, a very low value and for extremely long series an almost vanishing relative fluctuation. This decrease in the value of the fluctuation about the mean is well known and the application to the explanation of the Brownian movement and other phenomena is familiar to students of physics. The numerical results derived from the data of the experiment are interesting however, and the ease with which the smooth curve of Fig. 8 was drawn is suggestive.

If we sample the results by selecting at random a number of groups of 50 consecutive events each, we find that the experimental probability, the fluctuations, the sequences and the distributions of favorable and unfavorable cases are very different. If however, we take samples of 1,000 each we find that in some respects these samples are very much alike, in others very different. The experimental probabilities and the extreme fluctuations are each very much the same and for any "run of mine" thousands will be remarkably like the sample. If we take samples of 10,000 each, this statement is much more forcible. If the experiment had been extended so that samples of 100,000 or more could be taken, it is almost certain that the variation in  $P$  and in the range of fluctuations would be found to be almost nil.

There are however important differences in the distribution of favorable and unfavorable cases even in long sequences. For example, in one place there appeared a sequence of 13 favorable cases, followed almost immediately by 21 unfavorable cases. Another small series had eight singles, another four four's, another, a long row of alternate favorable and unfavorable cases. These are not duplicated and no doubt there are many other oddities. Thus provided the series is fairly long, we may expect certain aspects of a series to change but little, but other aspects in which each may be unique. Of course it may be argued that if the observations are carried to very great numbers, all of these singularities or oddities will repeat. No one can refute such a statement but it is equally impossible to refute the statement that all of these particular ones will not repeat.

There is of course the most probable combination of hits and misses in, say, 50 events, and this might be found many times. The peculiarities referred to are far less probable. That there would be a sequence of 13 passages followed almost immediately by 21 misses was very improbable. Any particular distribution may have a probability attached to it and if the probability is small enough we might be tempted to say that the distribution will not occur. But perhaps it did occur! And perhaps the particular distribution referred to came early!

That a sequence of 12 or 13 should appear anywhere in the experiment was most unexpected. Indeed, according to theory a 13 might be expected once in a million events, yet it appeared in the 21st thousand. The 12 appeared in the 49th thousand. Either might have appeared anywhere else, might indeed have occurred in the same thousand. There is nothing to distinguish the thousands and neither time nor place in a series has anything to do with the matter.

Again where perfect randomness obtains, what has happened has no influence on what may happen. This is contrary to an instinctive feeling which is common to most people, even the initiated. For example, if a coin is tossed repeatedly and heads has appeared, say, five times in succession, the exercise of a rather strong will would be necessary to enable one to call "heads" for the next throw. However it is as likely then as it ever was or ever will be and the

probability is one-half.\* The appearance of the 13 in the 19th thousand of the experiment described in this paper had no influence for or against the appearance of the 12 in the 49th thousand. The repetition of certain types of hands in cards and what is generally called a "run of luck" must be explained either as a result of lack of randomness or they may be just cases where improbable events appeared early in the series. (For modern bridge players they may not be very early.)

What about the appearance of the extremely improbable, say, a run of 1,000 in the experiment? The probability of such a sequence is  $0.3556^{1000}$ , a very small number. Might it appear? This question is getting near the core of a very important matter, *i.e.*, the occurrence in nature of an event which is said to be extremely improbable, but is really treated as though it were impossible.

The mathematician would certainly answer in the affirmative. Assuredly, if there is a probability that a phenomenon may take place, the possibility of its occurrence must be admitted. Any one who has watched the course of the experiments of this paper, and has seen thousands of balls dropped, finds it difficult to entertain the possibility of such an excessively long run. Feeling however is a very poor guide in answering the question and if the mathematical theory may be applied to such extreme cases, the possibility of runs of any length must be granted.

A dart with a needle point thrown at random on to a large target must find a resting place which is so small compared with the available space as to give for this resting place an almost infinitesimal probability. Here apparently, the extremely improbable must happen at each cast of the dart.

Or turn to the tossing of a coin. Suppose a perfect coin to be tossed 100,000 times. The probability for heads at each cast being  $\frac{1}{2}$ , the total number of times that heads appears should be approximately 50,000. So much we may predict with confidence. What about the distribution of heads and tails? We know absolutely nothing about it, save that before any cast the probability for head or tail is always  $\frac{1}{2}$ . Since this is true for every cast any configuration of the series is as likely as any other and the probability of any particular configuration is a number so small as to be almost zero. But it may occur and one of these highly improbable configurations must occur, improbable as it is.

That the extremely improbable happens in every day life is well known to everyone. All can recall phenomena which before they appeared would have had very low probabilities.†

\* It is almost certain that prolonged experiments with dice and coins would show a slight predilection for one face. The construction of an ordinary die even if perfectly made must displace the centre of gravity toward the "one" and may make the "six" appear oftener than it should. The same kind of criticism may be offered regarding a coin experiment. If it is true in either case that the centre of gravity is displaced ever so little there will be a few critical cases in a long series where this displacement will determine the way in which the die or coin will lie.

†The writer well remembers standing as one of a group of small boys looking eagerly aloft to try to discern a small steel arrow that had been shot vertically upward and which had disappeared from sight. The first knowledge which any of the group had of the return was the sound of it plunging into the breast pocket of a member of the group. If the probability of that destination had been calculated it would have been extremely small.

To return to the question. May the extremely improbable occur in nature and is there any limit to what may happen? If two bodies at different temperatures are placed in contact, the postulate underlying the second law of thermodynamics states that their temperatures will approach each other and ultimately will become equal. According to the work of Boltzman, Planck and others, processes of this kind go in a definite direction, always in the direction of greater probability, *i.e.*, the molecules interact in such a way that the final configuration is more probable than the initial. If it be a matter of probability only, is there the slightest hope that one might observe the warm body becoming warmer and the cold one colder?

Or is there a chance that all of the gas molecules in a small region in a child's balloon will move away from the wall at the same instant, causing a dimple to appear? Or does one expect to see the needle of a steam gauge move suddenly or a stone leap into the air? If two chambers containing different gases at the same temperature and pressure are put into communication, the gases mix. Will they ever unmix? Planck (3, p. 50) has discussed problems like some of these and has given the answer somewhat as follows.

Pure mechanical motions are reversible. The motions of individual atoms and molecules are pure mechanical motions and subject to dynamical laws. But the mixture of gases is highly reversible. Each individual particle acting on another performs pure mechanical motion and the motions of the pair are reversible. In the mass however the motions of a great number are irreversible. We are able to say nothing about individual molecules in a mass of gas. We cannot see the particles; we don't know where any one may go or what it may do. The limitation imposed on us because of our inability to deal with individual molecules drives us to probability methods. We say that the swarm of particles will go from a less probable to a more probable configuration and complete mixture is the result. We say that a reversal of the phenomenon is so improbable that it does not happen.

We may put a similar question. Why does entropy increase in natural processes and never decrease? The answer is exactly the same, only the question is put in a different form.

If we deal with two or three or any small number of particles, the differences between the probabilities of different configurations are small so that any possible configuration may appear and the actions of these particles are reversible. If two molecules say of nitrogen and two of oxygen mix without reaction, they may separate readily. When then does the reversible become irreversible? According to Planck, when the number of particles becomes large enough to yield a mean value of the variables such as pressure and temperature which define the state, the fluctuations become too small to be important, reversibility disappears, the phenomenon becomes irreversible and the entropy increases. Apparently then entropy may decrease but does not because of the improbability that it will do so.

The second law of thermodynamics has many aspects. It is in the first place a denial of the *possibility* of a natural process attended by a decrease in



entropy. It is also in a sense a confession of our limitations. If like Maxwell's demon we could handle individual particles, we could cause processes to reverse and entropy to decrease. The law does not say that decrease of entropy is highly improbable but rather emphatically denies the possibility. If we admit a residue of probability even though it is extremely small, we must admit the possibility of decrease of entropy where by the law we deny it. The writer is inclined to the view that the decrease is indeed impossible, not highly improbable—and that the law is correctly stated in terms of the impossible. How then may we retain probability in our scheme of things and deny the possibility of an event which will decrease the entropy? Perhaps we may pass from improbable to impossible over the same bridge which Boltzmann built from reversibility to irreversibility via the mean value. When the number of events becomes large enough and the fluctuations relatively small enough, *i.e.*, when a mean is attained and preserved, certain configurations become impossible and not simply improbable.

If the theory of probability may be applied without restriction or limitation we must admit the possibility of any event which may have a probability assigned to it no matter how small. On the other hand if we accept the second law of thermodynamics in the form in which it is usually stated we must deny the possibility of certain operations which according to modern theory are merely highly improbable.

Of course it may be that highly improbable events like those already mentioned have occurred but, because of their momentary character or their location in distant parts of the universe, have escaped notice. Or they may occur at any time—not necessarily in the distant future—and strange phenomena may take place. On the other hand, the application of probability may be limited and the second law may reign in undisputed sway over a domain in which these strange phenomena are impossible. The latter view seems more reasonable.

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## AN X-RAY ANALYSIS OF THE CRYSTAL STRUCTURE OF THE THALLIUM-TIN ALLOYS<sup>1</sup>

By H. J. C. IRETON<sup>2</sup>, J. P. BLEWETT<sup>3</sup> AND J. F. ALLEN<sup>4</sup>

### Abstract

An analysis of the crystal structure of thallium-tin alloys was carried out. It was found that the tin lattice constant had a maximum value at the eutectic point (42.5% thallium), while the superconducting curve showed a cusp-shaped minimum at this point and a peak at the solubility boundary. When tin was added to thallium a change from  $\alpha$  to  $\beta$ -thallium was observed. This change is similar to that already noted in other alloys of thallium.

### Introduction

The application of X-ray crystal analysis to alloy systems has proved a valuable complement to the thermal and microscopic methods which have been in use for many years. The three main conditions of alloys, namely, solid solutions, eutectic mixtures and chemical compounds are very well exhibited and differentiated by their X-ray diffraction patterns. In particular we note the following characteristics of X-ray patterns due to alloys:

(a) Solid solutions exhibit the pattern of only one component. This pattern suffers a small change in lattice parameter as the percentage composition is varied. It is evident that in this case the atoms of the "dissolved" metal replace the atoms of the other at the lattice points.

(b) Eutectic mixtures give unchanged patterns of both components superposed.

The analysis of the thallium-tin alloys was undertaken in an attempt to throw light on unusual superconductivity phenomena which they exhibited when investigated at low temperatures in this laboratory (1).

<sup>1</sup> Manuscript received August 22, 1933.

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<sup>4</sup> Graduate student, Toronto University, and holder of a fellowship under the National Research Council of Canada.



Comparison photographs were also obtained of the alloys mounted in the form of thin foils, with no supporting glass plate. These photographs showed that no detectable chemical reaction was due to the flour paste, and also indicated that the paste produced no characteristic diffraction lines.

### Results

A series of representative photographs is given in Fig. 2. Table I summarizes the readings taken and shows lattice constants obtained.

TABLE I  
LATTICE CONSTANTS OF THALLIUM ALLOYS

Thallium, %	Structure	Lattice constant, Å	
0	Double body-centered tetragonal	$a_0 = 5.818$	$c = 0.5455$
15	Double body-centered tetragonal Face-centered cubic	$a_0 = 5.820 \pm .005$ $a_0 = 4.833 \pm .004$	$c = .5455$
35	Double body-centered tetragonal Face-centered cubic	$a_0 = 5.822 \pm .006$ $a_0 = 4.835 \pm .008$	$c = .5455$
42*	Double body-centered tetragonal Face-centered cubic	$a_0 = 5.830 \pm .004$ $a_0 = 4.833 \pm .005$	$c = .5455$
58	Double body-centered tetragonal Face-centered cubic	$a_0 = 5.824 \pm .006$ $a_0 = 4.832 \pm .005$	$c = .5455$
70	Double body-centered tetragonal Face-centered cubic	$a_0 = 5.8$ $a_0 = 4.837 \pm .003$	$c = .54$
80	Face-centered cubic	$a_0 = 4.835 \pm .004$	
90	Face-centered cubic	$a_0 = 4.829 \pm .004$	
97	Face-centered cubic Hexagonal close-packed	$a_0 = 4.840 \pm .004$ $a_0 = 3.45$	$c = 1.60$
100	Hexagonal close-packed	$a_0 = 3.450$	$c = 1.60$

NOTE:—Radius of camera varied from 9.40 cm. to 9.46 cm. Wave-length of X-rays = 0.710 Å. Probable errors quoted throughout are average deviations.

\*Due to the importance of accuracy in the results obtained for this alloy, four photographs have been taken independently of different specimens of the alloy. The four results agree well among themselves and the above value is an average for the four photographs.

It was noted that  $\alpha$ -thallium, the allotropic form of thallium which is stable at ordinary temperatures and which displays a hexagonal close-packed structure, changes, upon the addition of tin, to  $\beta$ -thallium, which is ordinarily stable only above 231° C. (approximately). Both of these forms correspond to spherical symmetry in the atom, the change being accomplished by a simple gliding of planes. A similar change from  $\alpha$ - to  $\beta$ -thallium in the thallium-antimony alloys has previously been noted by Persson and Westgren (3).

The value of the lattice constant obtained for the alloy containing 90% of thallium agrees with that obtained by Sekito (4). The constant of the  $\beta$ -thallium lattice decreases to about 4.833 Å, corresponding to the interposition of the tin atom in the thallium cell. The lattice parameter of thallium-tin alloys containing less than 80% of thallium remains constant at about 4.833 Å throughout the eutectic phase. The constant for tin, on the other hand, undergoes a slight increase in the eutectic phase. This increase has its maximum at or near the eutectic point.

### Discussion

In general, the writers' observations on this series of alloys show no marked deviation from the laws describing the structure of alloy systems. However, an unexpected expansion of the tin lattice in the eutectic region has been noted. The value of this expansion is a trifle doubtful since it is not much greater than the experimental errors. It would seem probable that this phenomenon is related in some way to the lowering of the superconducting point. Since no adequate theory has yet been advanced to deal with superconductivity, the writers cannot further support this hypothesis.

The thermodynamic examination mentioned above has revealed another interesting feature of the eutectic region. In the cooling curves of the alloys between 0 and 82% of thallium a second stationary point appears below the point of eutectic crystallization. This stationary point is ascribed by Fuchs to the influence of the presence of tin on the point at which the change occurs from  $\beta$ -thallium to  $\alpha$ -thallium. His explanation is strongly supported when he traces the line  $AB$  (Fig. 1) which passes through these stationary points, through the solid-solution region to the point  $C$ , the point at which the change occurs for pure thallium. We would hence expect to find  $\alpha$ -thallium below the line  $AB$ , an expectation which is not fulfilled by the X-ray analysis.

The writers would suggest, since the transformation point lies below eutectic crystallization point, that the change which occurs in the thallium atom does not result in a change of crystal structure but succeeds merely in setting up strains resulting in a slight distortion of the tin lattice. This might appear in the form of an increase of the tin-lattice parameter such as has been observed above. Microphotometer traces have been taken which indicate that this distortion, if it exists, is not sufficient to produce a perceptible widening of the X-ray diffraction lines.

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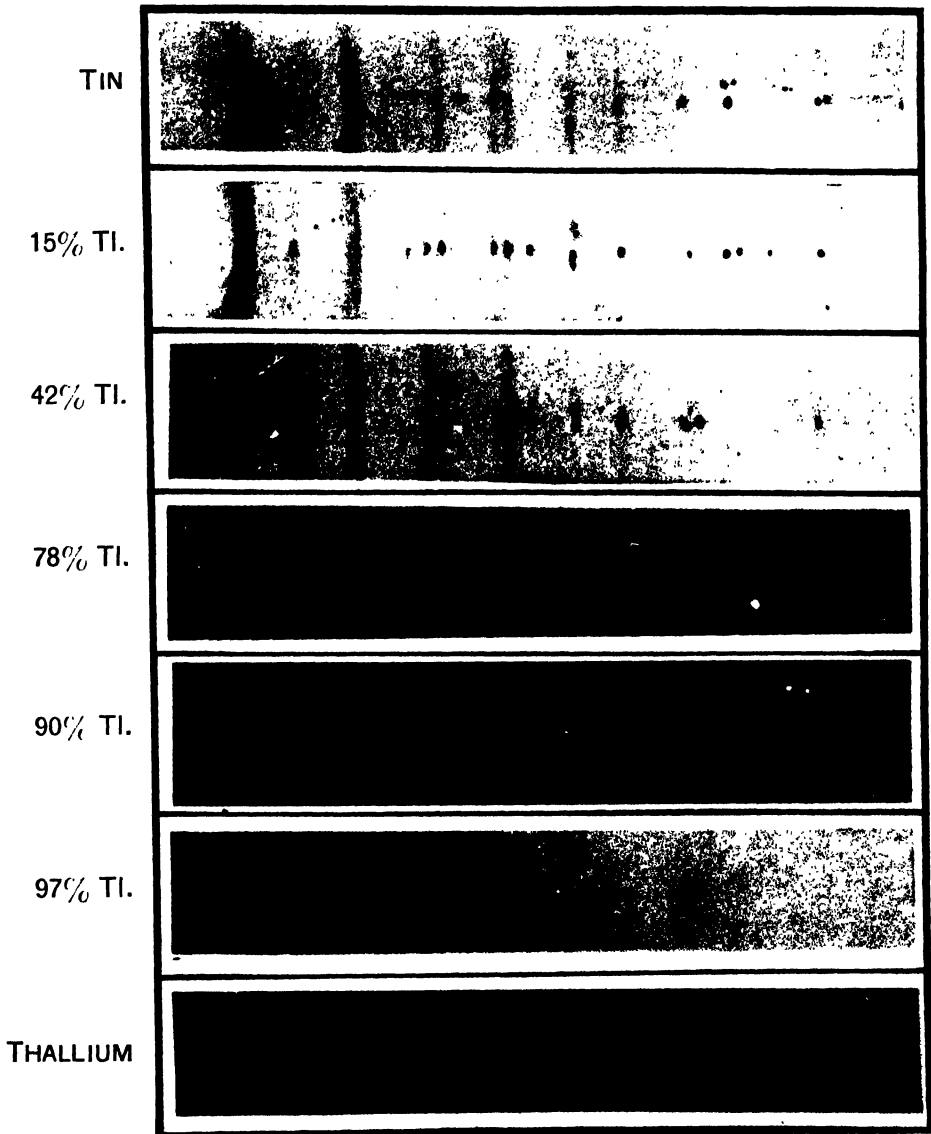


FIG. 2. Typical Hull-Debye-Scherrer X-ray diffraction spectrograms of thallium-tin alloys.



# THE VAPOR PRESSURE OF VINYL ACETATE<sup>1</sup>

BY J. MARSDEN<sup>2</sup> and A. C. CUTHBERTSON<sup>3</sup>

## Abstract

This paper presents the results of the measurement of the vapor pressure of vinyl acetate, over the temperature range from 0°C. to the normal boiling point. The determinations were carried out on vacuum distilled samples with an isoteniscope, differing slightly in detail from that used by Smith and Menzies(7).

The normal boiling point is 72.5°C. The molecular heat of evaporation has been found to be 8211 calories. The equation which represents the results is

$$\text{Log}_{10} P_{\text{mm.}} = \frac{-0.05223 \times 34433}{T} + 8.091.$$

Trouton's constant and the critical temperature have been found to be 23.8 and 228.3°C.

## Experimental

A diagram of the apparatus is shown in Fig. 1. The apparatus was pumped out at *P*. Vinyl acetate\* was admitted at *H* by opening a stopcock which was lubricated with glycerol and dextrose. The acetate which contained traces of acetaldehyde and acetic acid was distilled at room temperature into *G* which was suitably cooled with an ice-salt mixture. When the appropriate amount had distilled over, the tap connecting *G* and *H* was closed. On warming *G* and cooling *E*, a sample was distilled into the isoteniscope. A sufficient quantity was distilled to fill the apparatus including the carboy *C*, with vapor at a few centimetres pressure. The isoteniscope was then sealed off from the distilling apparatus. The limbs of the isoteniscope were about two-thirds full of liquid. The liquid was brought to constant temperature, accurate control of which was aided by brisk stirring and felt insulation on the water bath. On connecting the apparatus through the two-way stopcock *P* to the pump, the liquid began to boil in the bulb *F*. It was found however that without a capillary tube attached as shown at *F*

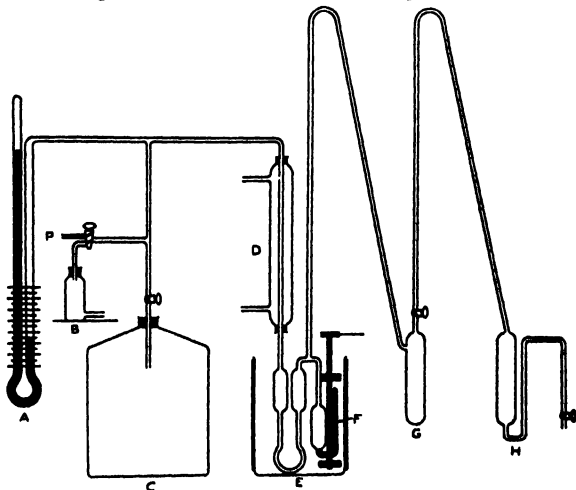


FIG. 1. Vapor pressure apparatus.

<sup>1</sup> Manuscript received May 11, 1933.

Contribution from the Department of Chemistry, Mount Allison University, Sackville, New Brunswick, Canada.

<sup>2</sup> Instructor in Chemistry, Mount Allison University.

<sup>3</sup> Assistant Professor of Chemistry, Mount Allison University.

\* Kindly supplied by Shawinigan Chemicals Co.



vaporization took place without ebullition. With the capillary however the surface was continually broken,—a necessary precaution to ensure equilibrium being established between liquid and vapor. In order to reduce loss during the boiling of the liquid, a reflux condenser *D* was added.

The vapor pressure was determined by connecting the apparatus to *B* and allowing dry air to enter slowly through a fine capillary which was attached to the lower end of the drying bottle. When the height of the liquid in

TABLE I  
VAPOR PRESSURE VALUES OF VINYL ACETATE

Temp., °C.	Press., cm.	Log press.	$1/T \times 10^3$	Temp., °C.	Press., cm.	Log press.	$1/T \times 10^3$
-0.15	3.19	.5032	3.665	38.50	20.69	1.3155	3.210
3.65	3.79	.5780	3.615	40.05	22.07	1.3438	3.195
5.23	4.02	.6042	3.595	42.17	24.07	1.3813	3.173
7.80	4.63	.6656	3.562	44.23	26.15	1.4190	3.152
10.70	5.44	.7353	3.531	46.49	28.72	1.4578	3.130
13.15	6.25	.7961	3.495	48.42	31.73	1.5014	3.112
15.93	7.18	.8561	3.462	51.64	35.13	1.5417	3.080
19.15	8.63	.9360	3.422	55.62	41.07	1.6135	3.043
21.07	9.46	.9759	3.401	57.20	43.94	1.6429	3.028
23.05	10.59	1.0249	3.378	59.00	46.96	1.6717	3.012
25.30	11.50	1.0607	3.352	61.32	50.97	1.7073	2.991
27.30	12.69	1.1033	3.330	64.25	56.74	1.7539	2.965
28.90	13.68	1.1360	3.312	66.62	61.73	1.7905	2.944
31.62	15.24	1.1829	3.283	68.90	66.63	1.8237	2.925
32.21	15.90	1.2011	3.277	72.10	74.37	1.8714	2.898
33.42	16.53	1.2183	3.263	72.50	76.00	1.8808	2.895
36.94	19.33	1.2862	3.225				

the two arms of the isoteniscope was level, the manometer reading gave the vapor pressure for a given temperature. The carboy *C* had a capacity of 51 litres which was sufficiently large to prevent sudden pressure changes and permitted the accurate control of the liquid movement in the limbs of the

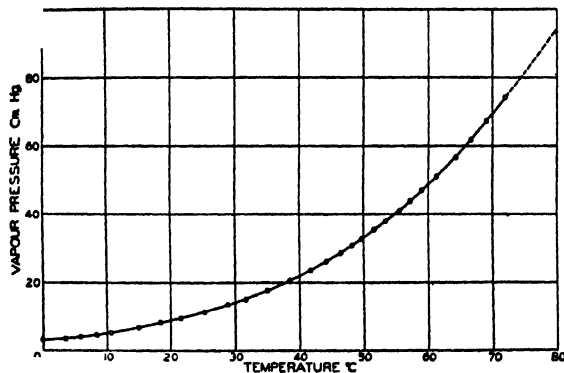


FIG. 2. Vapor pressure of vinyl acetate from 0° to 80°C.

isoteniscope. The values obtained for the vapor pressure are given in Table I. The logarithms of the pressures and the reciprocals of the absolute temperatures are also included.

The relation between pressure and temperature was plotted and is shown in Fig. 2.

On plotting the logarithm of the vapor pressure against the reciprocal of the absolute

temperature a straight line was obtained which permitted the calculation of the latent heat of evaporation and Trouton's constant. The equation which represents the results as plotted in Fig. 3 is

$$\text{Log}_{10} P_{\text{mm.}} = \frac{-0.05223 \times 34433}{T} + 8.091.$$

The value of the molecular heat of evaporation obtained from the slope of the line was found to be 8211 calories. Trouton's constant was calculated as 23.8.

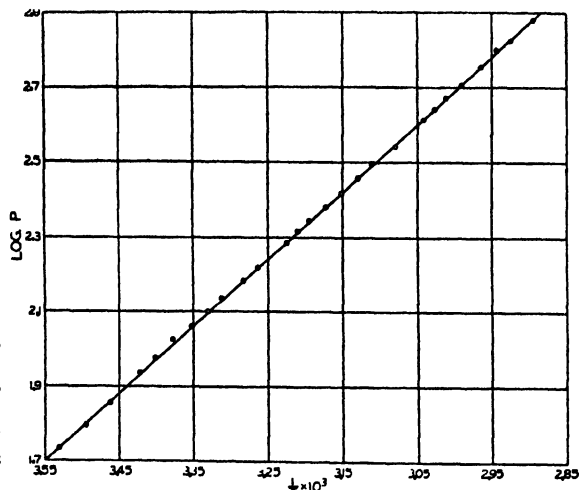


FIG. 3. Curve showing proportionality between the logarithm of the vapor pressure and the reciprocal of the absolute temperature.

### The Calculation of the Critical Temperature

In an attempt to get an approximate value for the critical temperature of vinyl acetate three methods have been employed and the average of the values calculated was taken as most representative.

Ramsay and Shields (5) give the following relation between critical temperature and surface tension;

$$\gamma \left( \frac{M}{d} \right)^{\frac{1}{3}} = k (t_c - t - 6),$$

where  $\gamma$  = surface tension;  $M$  = molecular weight;  $d$  = density at temperature  $t$ , and  $t_c$  = critical temperature. Using the surface tension values of Green, Marsden and Cuthbertson (1) at 20, 25 and 30°C. the average value for  $t_c$  was 228.9°C.

Ramsay and Young (6) pointed out that if the absolute boiling points of two closely related liquids  $A$  and  $B$  are compared under equal pressures the following relation will be found to hold,

$$\frac{T_A^c}{T_B^c} = \frac{T_A}{T_B} + c (T_A - T_B)$$

where  $T_A$  and  $T_B$  are the absolute boiling points of two similar liquids and  $T_A^c$  and  $T_B^c$  are the absolute critical temperatures of the liquids. The constant has been taken as zero.

Two liquids, ethyl propionate and normal propyl acetate, have been chosen as similar liquids. They have five carbon atoms in the molecule and the value of the Ramsay and Shields constant is 2.3.

TABLE II  
CRITICAL TEMPERATURES AND BOILING POINTS OF  
ETHYL PROPIONATE AND NORMAL PROPYL ACETATE

—	Critical temp., °C.	B.p., °C.
Ethyl propionate	272.9	99.10
Normal propyl acetate	276.2	101.7

Values for the critical temperatures and boiling points of these two compounds, taken from the International Critical Tables (2, p. 248) are given in Table II.

The critical temperature of vinyl acetate calculated from these data is 233.6°C.

Sugden (8) has shown that the equation  $D - d = D_o \left(1 - \frac{T}{T_c}\right)^{\frac{2}{15}}$  is trustworthy for the relation between density and temperature of unassociated liquids; where  $D$  = density of the liquid at absolute temperature  $T$ ;  $d$  = density of the vapor;  $D_o = \frac{M}{V_o}$ , where  $M$  = molecular weight and  $V_o$  = molecular volume at absolute zero.  $T_c$  is the absolute critical temperature. The zero volume may be calculated from atomic and structural constants. For vinyl acetate the value is 70.6. The values obtained at 10° and 30°C. give an average value of 222.4 for the critical temperature. The values calculated by the various methods are shown in Table III.

TABLE III  
CALCULATED CRITICAL TEMPERATURES

Method of calculation	Critical temp., °C.
Ramsay and Shields	228.9
Ramsay and Young	233.6
Sugden (zero volume)	222.4
Average	228.3

### Discussion of Results

In order to check the vapor pressure curve, the boiling point of air-distilled vinyl acetate was determined. At a corrected pressure of 74.35 cm. the liquid boiled at 72.2°C. The value as read from the vapor pressure-temperature curve (Fig. 2) is 72.25°C.

It would seem probable that, owing to polymerization, the vapor pressures of vinyl acetate at elevated temperatures would be considerably lower than those of the monomer. If this were so the value of the vapor pressure should be a function of the time of heating at higher temperatures. In order to check this possibility a fresh sample distilled *in vacuo* was raised to a temperature of 70°C. in 15 min. The value obtained agreed accurately with those obtained in the normal course of measurements which extended over two hours. Polymerization then appeared to be so slight that it had no perceptible influence on the vapor pressure. However, if samples stand in contact with air, a pronounced effect is noted. This effect can be accentuated by

bubbling oxygen through the liquid. One finds a marked increase in viscosity which is a sensitive criterion of polymerization. Care was taken to exclude oxygen from the isotenoscope, which probably accounts for the negligible effects produced at elevated temperatures.

The value of Trouton's constant (23.8 as compared to an average normal value of 21.7) might suggest an associated liquid. It was found that Ramsay and Shields' constant of 2.41 is higher than the accepted average value of 2.12 (1). It is notable that some acetates (3, p. 450; 4, p. 136) show about the same divergence for both constants.

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## THE ACTION OF CARBON DISULPHIDE ON ALUMINA GEL<sup>1</sup>

BY L. A. MUNRO<sup>2</sup> AND J. W. McCUBBIN<sup>3</sup>

### Abstract

The authors have investigated the yellow color observed when carbon disulphide was adsorbed by c.p. alumina at room temperature. The color is due to by-products of the reactions of carbon disulphide with residual water in the gel. The investigators of the  $\text{CS}_2 + \text{H}_2\text{O}$  reaction at higher temperature attribute the yellow to sulphur or aluminium sulphide. The color formed at room temperature is not due to either of these. The reaction products consist largely of hydrogen sulphide, water, and carbon dioxide, with small amounts of carbonyl sulphide and carbon monoxide. The yellow coloration has been found to be a mixture of sodium sulphide, sodium hydrosulphide, and sodium polysulphide. A mechanism is proposed for its formation.

### Introduction

During experiments on the adsorption of various vapors by alumina gel (13), the formation of a yellow color on the sorbent was noticed when carbon sulphide was used. This color could not be removed with the usual solvents for sulphur.

No references to the formation of a yellow coloration with carbon disulphide at room temperature, using alumina or any other sorbent, were found in the literature. Gurwitsch (6) mentions the evolution of hydrogen sulphide when carbon disulphide is adsorbed by fibrous alumina. He does not state the temperature at which this reaction was observed, the experimental technique, nor his method of analysis of the reaction products. No yellow coloration is recorded.

Simonin (17) and Allmand and Lizius (1) have measured the sorption of carbon disulphide by active charcoal at room temperature. They did not report any yellow deposit.

Various investigators have noted a yellow coloration at higher temperatures (2, 5, 14, 15). It has been shown that sulphur is formed at higher temperatures with some catalysts.

The present study was undertaken to ascertain the nature of the yellow coloration produced at room temperature, and the reactions by which it is formed.

### Experimental and Discussion

#### *The Gel*

Mallinckrodt's alumina gel was used in the original sorption study (13). Baker's product and that of the British Drug Houses also gave the yellow coloration. Both are doubtless prepared from bauxite.

A sample of prepared gel was also investigated. It was obtained by precipitation from a hot solution of aluminium nitrate with ammonium hydroxide. It was thoroughly washed and dried at 25° C. to a hard glass-like mass.

<sup>1</sup> Original manuscript received June 26, 1933.

Contribution from the Department of Chemistry, Queen's University, Kingston, Ont. Presented to the Royal Society of Canada, May, 1933.

<sup>2</sup> Assistant Professor of Chemistry, Queen's University, Kingston, Ont.

<sup>3</sup> Demonstrator, Department of Chemistry, Queen's University.

A careful analysis of Baker's gel gave the following figures:—water, 35.3%; silica and ferric oxide, traces; alkali metals, 0.06%; and the remainder, alumina. The analysis for the alkali metals was made according to the method outlined by Washington (20). As the amount was approximately equal to the experimental error involved in his method, qualitative microchemical tests were tried on a hot-water extract of the gel in platinum. These tests revealed the presence of sodium, which was combined with the hydroxyl radical. This was confirmed by a spectroscopic analysis, using a copper arc free from alkali.

Along with the lines from the copper arc and the bright ones of aluminium, faint lines for sodium and also fainter ones for calcium, magnesium, silicon, and iron were visible. Potassium was shown to be absent. Baker's gel was chosen as the catalyst in this investigation.

#### *Activation of the Catalyst*

Munro and Johnson (13) showed that the optimum temperature of activation for the sorption of ether vapor was 400° C., which left a residue of 4.5–7.2% of water in the gel. This temperature was confirmed by Chowdhury and Bagchi (3) as the optimum for the removal of sulphur compounds from oil by alumina gel. Therefore, 400° C. was chosen as the activation temperature in this study.

#### *Purification*

The c.p. carbon disulphide was purified by very slow reflux distillation through phosphorus pentoxide in an all-glass apparatus. Only the middle fraction was employed. This was redistilled just before use and kept over mercury. It had the characteristic sweet ethereal odor of pure carbon disulphide.

That the purified carbon disulphide was free from hydrogen sulphide was shown by different experiments. Nitrogen saturated with carbon disulphide was bubbled through a copper acetate solution for 48 hr. and there was no precipitation of copper sulphide. In a second experiment the carbon disulphide was bubbled through iodine solution for some time. There was no difference in the titrations before and after contact with carbon disulphide.

#### *Experimental Methods*

Dry carbon dioxide-free air was passed through a saturator of carbon disulphide and through the activated alumina. The saturator and the alumina were kept in an air bath at 25° C. Each experiment was stopped when the alumina tube had completely assumed the canary yellow color.

To make sure that the formation of the color was not due to oxidation, nitrogen, purified with pyrogallol made up according to the directions of Wolf and Krause (21), was used during activation and as carrier during sorption. There appeared to be no difference in the reaction, the yellow color appearing as usual.

Photochemical effects were also eliminated by carrying out distillation, activation, and sorption in the dark. The reaction proceeded as before.

### *Identification of the Reaction Products*

It was found early in the course of the work that hydrogen sulphide was given off as one of the reaction products. The only hydrogen present was that contained as water in the residual water in the gel. The conclusion was that this must be a reaction involving carbon disulphide and water, with perhaps a subsequent reaction with some impurity in the gel.

From the work of Neumann and Altmann (14), Stock, Siecke and Pohland (19) and others, it appeared that the reactions might result in the possible formation of carbon disulphide, carbon dioxide, carbonyl sulphide, carbon monoxide, carbon sulphide, hydrogen sulphide, water, sulphur dioxide and sulphur. A quantitative determination of the products was not necessary for the elucidation of a mechanism of the reactions, but rather identification of all the products. This was undertaken.

There is no method of analysis outlined in the literature for such a mixture. The methods given for smaller mixtures are vitiated by the presence of certain components of the larger mixture. Further, the methods given are for static determinations and could not be used dynamically without modification. An account of the examination of the possible reaction products, together with the conclusions drawn, are given below.

**Sulphur.** The fact that the yellow color was not removed by solvents for sulphur precludes the presence of free sulphur unless in an insoluble modification. As the reaction takes place at room temperature this seemed highly improbable.

**Hydrogen Sulphide and Sulphur Dioxide.** It was observed early in the investigation that the formation of the yellow color was accompanied by the evolution of hydrogen sulphide. For the detection of sulphur dioxide, the sulphide was completely precipitated by bubbling the exit gases through cadmium acetate solution and the filtrate tested for sulphur dioxide with fuschin. Negative results were obtained. Sulphur dioxide was also absent in the desorbed gases.

After proof of the absence of sulphur dioxide, hydrogen sulphide was shown to be present in large amounts by means of an iodine solution in potassium iodide. Carbonyl sulphide does not react with this reagent. With the salt of a heavy metal, however, it gradually precipitates the sulphide.

**Carbon Dioxide.** Carbon dioxide could not be identified by the usual methods owing to the interference by carbon disulphide and carbonyl sulphide which react with alkali with the formation of carbonates and thiocarbonates. Identification can be made by taking advantage of the considerable difference in rates of absorption of these gases or vapors in alkali of different concentrations. Stock and Seelig (18, p. 676) give the following method for the analysis of a mixture of carbon monoxide, carbon dioxide, carbonyl sulphide and carbon disulphide:—(a) Sodium hydroxide (1 cc. of 30%) is introduced into the burette. The volume is read each minute for several minutes. A rapid shrinkage shows the presence of carbon dioxide. (b) Water (4 cc.)

is added, and the volume read every 10 min. for one hour (carbonyl sulphide determination). (c) Potassium hydroxide (1 cc. of 30%) is added and the volume recorded after one, two and three days (carbon disulphide determination). (d) The residual gas (carbon monoxide) is determined by cuprous chloride.

After the removal of hydrogen sulphide the remaining products, with the exception of carbon monoxide, were condensed in liquid air and examined by the above method. An immediate rapid shrinkage in volume was noted. This could only be carbon dioxide.

**Carbonyl Sulphide.** Fig. 1 shows the absorption curves for (b), for two different experiments. The absorption of carbon disulphide from a saturated air-carbon disulphide mixture is shown in curve No. 3. It will be seen that in approximately one hour the rates in curves Nos. 1 and 2 become similar to that of No. 3. From these and other experiments it was concluded that some carbonyl sulphide was present in the reaction products.

**Carbon Disulphide.** Carbon disulphide was always present as the result of incomplete reaction.

**Carbon Monosulphide.** The existence of gaseous carbon monosulphide has been questioned although the brown polymer is known (9). Lewis and Lacey (10) suspected its formation in their investigation of the equilibrium  $\text{CO} + \text{S} \rightarrow \text{COS}$ , but they did nothing to confirm this. The writers have found no evidence of either form in the reaction products.

**Carbon Monoxide.** Any carbon monoxide formed would pass through the liquid-air trap. The residual gases were analyzed for carbon monoxide in a Fisher Universal gas analysis apparatus. The carbon monoxide was passed over heated copper oxide and the carbon dioxide absorbed in caustic. Carbon monoxide was found to be present in small amounts.

**Water.** It was found that under ordinary conditions water was absent from the exit gases, owing to adsorption by the active gel. It was detected in the reaction products after reaction had gone on for some time, or when the activation temperature was lowered to 150° C.

#### Identification of the Yellow Coloration

It has been shown above that the yellow coloration is not sulphur in its ordinary form. Neumann and Altmann (14) suggest that, during the reaction of carbon disulphide on water vapor in the presence of alumina, aluminium sulphide is formed as an intermediate, and is then hydrolyzed by the water to form alumina and hydrogen sulphide. Accordingly, if water were used to

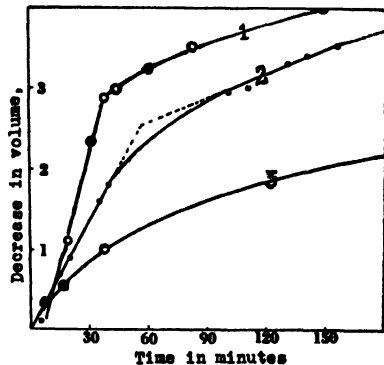


FIG. 1. Rate of absorption by 6% sodium hydroxide. Curves 1 and 2. Condensates by liquid air. Curve 3. Carbon disulphide from saturated air mixture.



extract the gel, the yellow color would disappear owing to the reaction stated above. This was tried on the yellow gel, and contrary to expectations, although the gel became white, the aqueous extract was a clear yellow.

Qualitative analysis by micro methods showed that this solution contained sodium combined with sulphide, hydrosulphide and some polysulphide. The sodium was detected by the formation of the characteristic tetrahedra and octahedra of sodium uranyl acetate. After boiling for 10 min. hydrogen sulphide was still present in the vapor, indicating the hydrolysis of a hydrosulphide. The test of Rule and Thomas (16) for sodium disulphide was positive.

The gummy residue, left on evaporation of the water extract of the yellow coloring matter in a stream of nitrogen, revealed the bright sodium doublet 3302.5 and 3302.94 Å, as well as the very sensitive magnesium line 2852.12 Å. However a negative test for magnesium (sensitivity 1/100,000) as outlined by Feigl (4, p. 245) showed that it was present in traces only.

From the foregoing it is concluded that the following products are formed in the reaction: hydrogen sulphide—in the largest quantities—water, carbon dioxide, and small amounts of carbonyl sulphide and carbon monoxide. The gel contains as a yellow coloration a mixture of sodium sulphide, sodium hydrosulphide and a small amount of sodium polysulphide.

#### *Proposed Mechanism of the Reactions*

The primary reaction is one between carbon disulphide and water. These may react in two ways,



According to Neumann and Altmann (14) reaction No. 2 seems to predominate at lower temperatures. A sufficient number of thermochemical data are not available to determine by that means which of these two reactions will predominate at room temperature.

Carbonyl sulphide reacts with water according to the equation:



Stock and Seelig (18) have studied the decomposition of carbonyl sulphide, and they state that it breaks up according to the equation



The investigation of Lochte-Holtgreven and Bawn (11) on the structure and decomposition of carbonyl sulphide led them to believe that the sulphur formed in this decomposition (No. 4) is an excited sulphur atom with excitation energy of 35 cal. Although there was no investigation into the reactivity of this sulphur, it might be that it would combine much more readily with a sulphide to form a polysulphide.

Analysis has shown that there is a trace of sodium hydroxide in the gel. The hydrogen sulphide reacts with this to form sodium hydrosulphide:



The sulphur from the decomposition of carbonyl sulphide may now react with the sodium hydrosulphide to form the polysulphide.



It will be seen from this that hydrogen sulphide is produced in the largest quantities. The writers found the same to be true experimentally.

#### *Further Investigation to Support Proposed Mechanism*

To see whether the yellow coloration was being caused as the result of a secondary reaction between the hydrogen sulphide and the trace of alkali in the gel, dry hydrogen sulphide was passed through activated alumina at room temperature. A calcium chloride tube was placed directly after the alumina to detect the presence of water formed in the reaction.

The yellow color formed in the tube as before, but with a gradation from an intense yellow at the entrance end to white at the exit end. As shown by equation No. 5 water is given off as the result of the reaction. Some of this water formed is taken up by the alumina, preventing adsorption of the hydrogen sulphide, and therefore preventing the reaction in which the yellow coloring matter is formed. After saturation the water emerged and was absorbed by the calcium chloride. These results are recorded in Table I.

TABLE I  
SORPTION OF HYDROGEN SULPHIDE

Vol. of H <sub>2</sub> S supplied, S.T.P., cc.	165	235	375	540	627
Weight of H <sub>2</sub> S supplied, gm.	0.2504	0.3567	0.5692	0.8197	0.9513
Gain in weight of Al <sub>2</sub> O <sub>3</sub> , gm.	0.1304	0.1963	0.3235	0.4505	0.4741
Gain in wt. of CaCl <sub>2</sub> , i.e., loss of water from gel	Nil	Nil	Nil	0.0020	0.0063

NOTE:—Adsorbent, 17.240 gm.; residual water, 7.0%; activation temp., 375° C.

Carbonyl sulphide reacts with sodium hydroxide, but the reaction is rather slow. It also reacts very slowly with water to form hydrogen sulphide and since, according to the proposed mechanism, the formation of the yellow color depends on the initial production of hydrogen sulphide, it was decided to investigate the action of pure carbonyl sulphide on the active alumina.

Carbonyl sulphide was prepared by the action of dilute sulphuric acid on potassium thiocyanate (8). It was purified by bubbling through 30% sodium hydroxide, iodine solution, then through a condensing worm immersed in solid carbon dioxide and chloroform, and thence through the alumina. A faint coloration appeared throughout the gel.

This is what would be expected from equation No. 3. It may be concluded that this reaction goes to a very slight degree at room temperature. However, on standing for some time the gel became much more yellow, with the evolution of hydrogen sulphide.

### Purification of the Gel

It was thought that the sodium hydroxide in the gel might be removed by electrodialysis. A modified form of the dialysis cell as described by Holmes (7) was used. Mechanical stirring proved to be much more efficient than the compressed air method described by Holmes. Heat was supplied to the cell by means of a lamp enclosed in an asbestos box under the cell. Platinum gauze electrodes were substituted for the carbon ones recommended. A potential of 110 volts was used.

A drop from 20 to 0.6 milliamperes occurred in the first two days, and thereafter the current remained constant at the low amperage. The dialysis was continued for six weeks and the gel was then removed. Even after this time the gel gave the same coloration after reaction with carbon disulphide.

Regarding the removal of the electrolytes from gels, Lottermoser (12) states, "Absolutely electrolyte-free, pure silica sol cannot be prepared."

### Use of Extracted Gel

Gel that had been saturated with carbon disulphide was washed thoroughly with distilled water, dried at 110° C., and reactivated at 400° C.

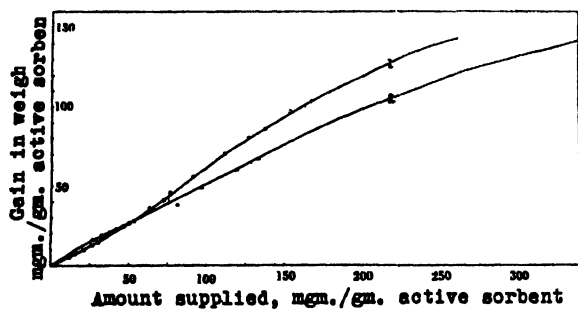


FIG. 2. Sorption by original and extracted gels. Curve 1. Sorption by original gel. Curve 2. Sorption by gel after extraction of yellow color and reactivation.

The residual water in the original sorbent was 9.2%; in the reactivated gel 9.9%. On passing carbon disulphide vapor through this gel no coloration developed, but hydrogen sulphide was given off. Sorption curves are shown in Fig. 2. The aqueous extract of this saturated gel gave no microchemical test for sodium. This fact supports the proposed mechanism.

### Acknowledgments

The authors wish to acknowledge their indebtedness to Queen's University for an assistantship to one of them (J. W. M.); and to the departments which helped with spectrum analysis and photography.

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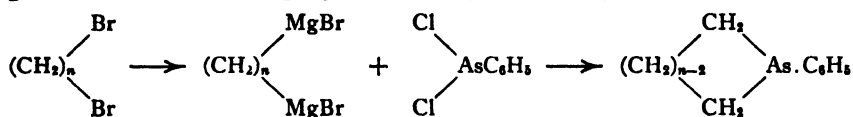
# STUDIES ON SOME UNSYMMETRICAL TETRAMETHYLENE GLYCOLS<sup>1</sup>

By C. F. H. ALLEN<sup>2</sup>, C. V. WILSON<sup>3</sup> AND W. L. BALL<sup>3</sup>

## Abstract

As intermediates for obtaining unsymmetrical 1,4-dibromides several tetramethylene glycols have been prepared, using varied procedures. They are not tractable substances. Only one gave a dibromide that could be purified; the latter did not form a Grignard reagent when treated with magnesium in dry ether. Trimethylene chlorobromide gives a small amount of Grignard reagent.

Heterocyclic compounds in which one of the atoms forming a part of the ring is arsenic have been prepared through the Grignard reaction (4, 5, 11).



The writers wished to study some of these heterocyclic compounds that were unsymmetrical, the preparation of which would require branched chain dibromides; only one of the latter was found in the literature (6, 13). The corresponding glycol,  $\beta$ -methylbutanediol-1,4, was previously known (6), but the necessary reactions were tedious and slow, and the yield low, considering the expensive starting material. A cheaper method was devised but the final yield was no better: citric acid  $\rightarrow$  itaconic acid  $\rightarrow$  methylsuccinic acid  $\rightarrow$  methyl succinic ester  $\rightarrow$   $\beta$ -methylbutanediol.

$\beta$ -Phenylbutanediol-1,4 was obtained by a sodium-absolute alcohol reduction of phenylsuccinic methyl and ethyl esters. While this work was in progress, Manske (7) described this glycol, prepared by an almost identical procedure, as an oil. In the writers' experiments a monosodium glycolate was always isolated and on acidification, the glycol was obtained as a crystalline solid. A homologue,  $\beta$ -(2,4-dimethoxyphenyl)-butanediol-1,4, was made by a similar reduction of the corresponding succinic ester.

Only the  $\beta$ -methylated glycol gave a dibromide that could be obtained in a pure state; the tendency to dehydration or removal of hydrogen bromide was very marked, the only derivative of the dimethoxyglycol that could be prepared being a diacetate.  $\beta$ -Methyltetramethylenedibromide-1,4 reacted with magnesium with formation of magnesium bromide, but if any Grignard reagent was formed the amount was too small to be detected.

A quantitative study of the formation of Grignard reagents from dihalides has not yet been made, but based on yields of products actually obtained, the tetra- and pentamethylene dibromides must have given 25-40% (4, 5,

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Contribution from the Department of Chemistry, McGill University, Montreal, Canada.

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11, 13, 14). The trimethylene gave under 1% (15) and ethylene dibromide and magnesium are said to yield ethylene "exclusively". It was thought that by using trimethylene chlorobromide the less reactive chlorine might allow the formation of a Grignard reagent, although not enough was obtained from ethylene chlorobromide to give Gilman's color test (3). A positive result was obtained, but the yield of Grignard reagent was too low to be practical (8%). This suggests that better yields might be obtained with higher mixed dihalides.

### Experimental

*$\beta$ -Phenylbutanediol-1,4.* This reduction was carried out essentially according to the published procedure, but the process of isolation differed. After refluxing had been completed, 300 cc. of water was added and the bulk of the alcohol distilled through a short column; when the temperature of the vapor reached 95° C. the heating was stopped. If allowed to stand overnight, flaky crystals separated, but as these were extremely difficult to filter, benzene was added and the lower layer drawn off; the solid that separated between the two layers was easily filtered. This substance is a sodium salt of the glycol and absorbs carbon dioxide rapidly. On acidification and extraction of the solution with benzene, followed by drying and distillation, the glycol was readily obtained as an oil (b.p. 158–60° C. at 8 mm.) which crystallized on cooling. After recrystallization from benzene or ether-petroleum ether it formed hexagonal plates, m.p. 70° C. Usually, to save time, the residual solution after removal of the alcohol was acidified at once and the oil taken up in benzene. The latter was shaken with sodium carbonate solution to remove a little phenyl succinic acid, then dried and distilled. The first crystalline material did not separate for several weeks, but once available for seeding, all the oils could be crystallized. The yield of sodium salt was 30 gm. from 62.5 gm. of ester, which gave 16–18 gm. of glycol (40–45%). The oily residues were combined and worked up for more glycol. The yield was not increased by using the methyl ester or substituting butyl alcohol. The sodium salt is a hygroscopic white solid, that absorbs carbon dioxide rapidly. Analysis:—Calcd. for  $C_{11}H_{13}O_2Na \cdot C_2H_6O$ : Na, 9.8%. Found: Na, 9.6, 9.7%.

The bis-phenylurethane was prepared in the usual manner; it melted at 114° C. and a mixed melting point with an authentic specimen was not depressed\*. The dibromide was never obtained in a pure state. Several procedures were employed including Manske's, but in every case an oil (b.p. 130–168° C. at 8 mm.) resulted. It was acid to litmus, liberated gas with methyl magnesium iodide, and contained 35% bromine (calcd., 54.8% Br). Obviously, it was unsuited for making a Grignard reagent.

*$\beta$ -(2,4-Dimethoxyphenyl)butanediol-1,4.* This glycol was also prepared by a sodium-alcohol reduction of the corresponding ester. The latter was obtained in a yield of 80% by refluxing for 10 hr. a mixture of 90 gm. of the

\*This substance had been prepared by Dr. Manske, and the authors are indebted to him for the mixed melting point determination.

corresponding anhydride (from resorcinol dimethyl ether\*, maleic anhydride and anhydrous aluminium chloride (9)), 200 gm. of methyl alcohol and 48 gm. of concentrated sulphuric acid, and distilling *in vacuo* (m.p., 62° C.). The reduction was carried out in the usual manner. After removal of the excess alcohol, benzene was added. On cooling, the glycol often crystallized. It separated from ether in fine prisms: m.p. 89° C.; yield, 50%. Analysis:—Calcd. for  $C_{12}H_{18}O_4$ : C, 63.7; H, 7.9;  $OCH_3$ , 27.4%; mol. wt. 226. Found: C, 63.9; H, 8.0;  $OCH_3$ , 27.5; mol. wt., 216.

This glycol† did not form a single solid derivative; it lost water on treatment with phenyl and  $\alpha$ -naphthyl isocyanates, *p*-chlorobenzoyl and 3,5-dinitrobenzoyl chlorides, and was recovered unchanged after treatment with trityl chloride in pyridine. It gave a liquid diacetate with acetyl chloride: 9 gm. of the glycol was dissolved in 20 cc. of acetyl chloride; hydrogen chloride was copiously evolved. The excess solvent was allowed to evaporate and the residue taken up in benzene, dried with calcium chloride and distilled *in vacuo*; 10 gm. of the ester (b.p. 221–4° C. at 18 mm.) was obtained. Analysis:—0.5525 gm. gave 0.2152 gm.  $C_2H_5O_2$ . Calcd., 0.2140 gm. On hydrolysis the glycol was regenerated.

No dibromide could be prepared by any method; the crude oil from various attempts analyzed 36.6% Br (calcd., 45.5%) but lost hydrogen bromide spontaneously. It boiled from 60–140° C. at 5 mm. and the distillate contained only 8% Br.

**$\beta$ -Methylbutanediol-1,4.** This glycol was prepared by the reduction of methyl succinic ester using butyl alcohol and sodium. After the addition of water the mixture was cooled and carefully neutralized with hydrochloric acid, the upper layer separated and the butyl alcohol distilled *in vacuo*. The residue from a run using 38 gm. of ester was extracted with ether and after removal of the solvent was distilled (b.p. 112–4° C. at 10 mm.), but when redistilled at 8 mm. gave two fractions: (a) 6 gm. (26%), b.p. 100–110° C. which was mainly the desired glycol; and (b) b.p. 135° C.; the refractive index of the latter was 1.4322<sub>D</sub><sup>20</sup>. The refractive index of 2-methyl-2-butene-1,4-diol (b.p. 128° C. at 7 mm.) is 1.4815<sub>D</sub><sup>20</sup> (10). From the first a bis-phenylurethane was prepared, having a melting point 99–100° C. (6).

The dibromide was prepared as directed by Harries (6), but the yield claimed could not be duplicated, nor was any better result obtained by other procedures; from 10 gm. of glycol was obtained 4–6 gm. of dibromide. The latter did not react with magnesium in dry ether without a crystal of iodine or Gilman's catalyst (2). After refluxing for 2.5 hr. much of the metal was unacted upon. White crystals of magnesium bromide etherate (8, 12) separated. Gilman's color test (3) was negative. No acid was produced on carbonation, nor could a mercuri-halide be prepared.

\*A liberal sample of this was kindly donated by E. I. DuPont de Nemours Co.

†When it was treated with *p*-chlorobenzoylchloride in pyridine, a white solid m.p. 195–96° C. resulted, which on analysis gave 24.1% chlorine. It was found to be *p*-chlorobenzoic anhydride (calcd. for  $C_{11}H_9O_2Cl$ ; Cl, 24.1%), yielding *p*-chlorobenzoic acid quantitatively on hydrolysis. The glycol was recovered unchanged.

*Bromobutyl acetate.* An attempt to form a dibromide from butyleneglycol diacetate ( $\begin{array}{c} \text{CH}_3\text{CHOCOCH}_3 \\ | \\ \text{CH}_2\text{CH}_2\text{OCOCH}_3 \end{array}$ ) was unsuccessful, only one bromine being introduced. Butylene glycol diacetate\* (50 gm.) was saturated with hydrogen bromide (50 gm. increase in weight) and after several hours poured upon cracked ice. The oil was extracted with ether, purified in the usual manner and dried over potassium carbonate. It gave a colorless liquid on vacuum distillation (b.p. 92–4° C. at 27 mm.). Analysis:—Calcd. for  $\text{C}_6\text{H}_{14}\text{O}_2$  Br: Br, 41.0%. Found: Br, 40.7, 41.2%. The ester was recovered unchanged after again saturating with hydrogen bromide; the location of the bromine was not determined.

In view of the recent success in reduction of esters to alcohols at very high pressures, using a copper-chromite catalyst (1), it was hoped that the glycols could be produced in this manner in a much better yield. Unfortunately the reaction proceeded too far, resulting mainly in a branched chain primary alcohol or cleavage.†

*Trimethylene chlorobromide and magnesium.* Trimethylene chlorobromide usually did not react with magnesium in dry ether without a catalyst. In one instance, after carbonation in the usual manner, the total acid was found to be equivalent to 8% of the halide used; glutaric acid was identified by a mixed melting point determination, and the characteristic disagreeable odor of  $\gamma$ -chlorobutyric acid was very noticeable.

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\*This substance was donated by Shawinigan Chemicals Limited.

†The writers are indebted to Professor Adkins of the University of Wisconsin for carrying out these reductions.



## THE ALKALOIDS OF FUMARIACEOUS PLANTS

VIII. *CORYDALIS AUREA*, WILLD. AND THE CONSTITUTION OF BICUCINE<sup>1</sup>BY R. H. F. MANSKE<sup>2</sup>

## Abstract

The chemical examination of the alkaloids of *Corydalis aurea* has shown an unusual complexity and of the total of more than ten alkaloids thus far isolated only six are now described. The record deals chiefly with the stems and leaves of the plant in which protopine was present in exceptionally low concentration (0.025%). Equally exceptional is its high concentration in the roots (1.6%). 1-Tetrahydropalmatine constituted the largest fraction of the remaining alkaloids, and its present isolation is the first on record although the *d*-form was previously known. Two new and well-characterized alkaloids, which have been named *capaurine* and *capauridine*, respectively, are isomeric and are best represented by the empirical formula  $C_{21}H_{27}O_5N$ . Both contain one phenolic hydroxyl and four methoxyl groups, and yield on methylation non-phenolic bases which do not appear to be identical. The presence of two bases, bicuculline and bicucine, which were first recorded in this series of papers, has again been demonstrated.

The constitutional analysis of the new bases, as well as the isolation and characterization of the minor alkaloids, is in progress.

In an appended note it is shown that bicuculline and bicucine are closely related and interconvertible. The latter is the free  $\gamma$ -hydroxy acid of which the former is the lactone.

With the exception of *Corydalis sempervirens*, *C. aurea*, Willd. (*Capnoides aureum* (Wild.) Kuntze) is the only species of this genus which extends its natural habitat to central and eastern Canada. According to several botanists whom the writer has consulted it grows well only on burnt areas or recent clearings, and it was in such habitats that the material for the present investigation was found. It was collected late in 1933, the glaucous blue foliage having partly turned to a dull reddish-yellow. The roots were severed from the aerial parts before drying and examined separately.

Owing to the fact that the contained alkaloid mixture is extremely complex, no fewer than ten apparently pure bases having been thus far isolated with the certain indication of still others, it seemed advisable to place on record some of the information obtained to date. Furthermore, a second lot of plant material is in process of examination and it is hoped that the combined mother liquors from the two will yield not only new bases but sufficient quantities of those present only in traces for adequate characterization.

Heyl (1) reported the presence of protopine and a second base (melting at 148-149° C.) in the roots of *C. aurea*, and this seems to be the only investigation on record concerning the plant. Although the roots have not been adequately investigated no indication of Heyl's second alkaloid has been found in the stems and leaves. Protopine was present in the dried roots to the astonishing amount of 1.6% while its presence in the stems and leaves was limited to no more than 0.025%, this in spite of a total alkaloid content

<sup>1</sup> Manuscript received September 29, 1933.

<sup>2</sup> Contribution from the National Research Laboratories, Ottawa, Canada.

<sup>3</sup> Associate Research Chemist, National Research Laboratories, Ottawa.

of at least 3.0%. The constituent present in greatest amount in the plant however is *l*-tetrahydropalmatine. The *d*-form of this alkaloid was isolated from *C. tuberosa* and recognized as such by Späth, Mosettig and Tröthandl (5) who suggested its identity with a number of bases obtained by several investigators from various sources. Owing to the present discovery of the *l*-form an element of doubt is raised in some of these cases. Two other bases which were first obtained from *Dicentra cucullaria* (2) but which have since become more familiar, namely, bicuculline and bicucine, were present in tractable amounts.

Only two of the six or seven remaining alkaloids which have been obtained from this plant have been adequately characterized. They are isomeric and analyses are in agreement with the formula  $C_{21}H_{27}O_5N$ . Both are phenolic (one hydroxyl) and contain four methoxy groups. Inasmuch as they appear to be new the names *capaurine* and *capauridine* are proposed, the first syllables being derived from the generic *Capnoides* Adans. and the remaining syllables from the specific name. Capaurine was obtained to the extent of 0.33% in large, stout pale yellow prisms, appreciably soluble in hot methanol and melting at 164° C.\* On methylation with diazomethane a non-phenolic base,  $C_{22}H_{29}O_5N$ , containing five methoxyl groups was obtained.

Capauridine was obtained to the extent of 0.018% in colorless fine needles practically insoluble in boiling methanol and melting at 204° C. Methylation does not yield the same methyl ether as that furnished by capaurine, though presumably the two are isomeric.

### Experimental

Throughout this investigation it was found advantageous to follow strictly the procedure detailed in a communication dealing with *Adlumia fungosa* (4), and in the following record the designations of the various fractions have the same significance. There was available 6.5 kilos of the dried stems and leaves, and except where specified the roots are not included in the following.

#### Isolation of Protopine

The precipitate (BS) consisted of a dark amorphous mass weighing only 3 gm. An appreciable amount of inorganic matter was removed by filtering a chloroform solution of this through a layer of charcoal. Evaporation of this to a syrup and addition of hot methanol and a nucleus yielded pale yellow crystals of protopine which, when recrystallized as hydrobromide, regenerated and again recrystallized, melted at 211° C., either alone or in admixture with an authentic specimen. An identical treatment of 195 gm. of the roots yield 3.2 gm. of once-recrystallized protopine, which after two further recrystallizations weighed 3.0 gm.

#### Isolation of *l*-Tetrahydropalmatine

The solution (SR) on slight cooling yielded a copious crop of pale yellow crystals. This was filtered off, washed with water and with ether (LC) and recrystallized from hot water. More of this salt was obtained from the com-

\*All melting points are corrected.

bined mother liquors (ASR) and a further quantity from the non-phenolic bases (BC). Repeated recrystallization yielded almost colorless needles melting at 232° C. to an orange melt, some sintering taking place at about 220° C.

A small portion of this was dissolved in hot water and cautiously treated with ammonia. The caseous precipitate rapidly crystallized. It was filtered off, washed with water, dried, and recrystallized from hot methanol in which it is moderately soluble. Large, stout, flat prisms with pyramidal terminations were thus obtained. The melting point is sharp at 142° C. In concentrated sulphuric acid it dissolves to a colorless solution which on heating becomes dirty greenish-brown and then turns to a deep brilliant purple which becomes red on dilution with water. Most characteristic is the fact that the crystals are triboluminescent, a livid blue light being emitted when they are rubbed with a glass rod in a dark room. These observations together with the analytical figures are sufficient to identify the base as tetrahydropalmatine. The optical rotation  $[\alpha]_D^{25}$ ,  $-278^\circ$  ( $c = 2.00$  in 95% ethanol) characterizes it as the *l*-form. Oxidized with iodine in alcoholic solution it yielded the sparingly soluble palmatine iodide melting at 243° C. Calcd. for  $C_{21}H_{25}O_4N$ : C, 70.99; H, 7.04; N, 3.94; 4 OMe, 34.93%. Found: C, 70.83; H, 6.82; N, 4.26; OMe, 34.87%.

Since *dl*-tetrahydropalmatine melts at 144° C. an equal mixture of the two forms should melt at a temperature not less than 142° C., the melting point of each form. In accordance with this supposition, a mixture of approximately equal amounts melted not quite sharply at 142–144° C. when slowly heated. The author is greatly indebted to Professor Ernst Späth of the University of Vienna for this specimen of natural *d*-tetrahydropalmatine.

#### *Isolation of Capaurine and Capauridine*

The precipitate (BCE) consisted of 27 gm. of an easily filterable granular product. It was suspended in 200 cc. of methanol and the mixture boiled for some time. After thorough cooling the separated crystalline material together with that not originally dissolved was filtered off and washed with cold methanol. This was dissolved in hot chloroform and the filtered solution (charcoal) evaporated to a syrup, and then repeatedly evaporated with fresh portions of methanol until crystallization in the hot liquid began. The colorless crop of fine crystals was filtered off and washed with cold methanol. As thus obtained *capauridine* melts at 203–204° C. some darkening taking place at 180–190° C. Recrystallization from chloroform-methanol did not improve the appearance, nor did it raise the melting point above 204° C. With concentrated nitric acid an intense cherry-red color was immediately developed. Calcd. for  $C_{21}H_{27}O_5N$ : C, 67.56; H, 7.24; N, 3.67; 4 OMe, 33.24%. Found: C, 67.64; H, 6.77; N, 4.08; OMe, 33.05% (mean of duplicates). This formula is at present preferred to the one with two hydrogen atoms fewer, because such a formula would almost certainly require an aporphine or protoberberine structure with five oxygen substituents, not a single

representative of which is known. On the other hand, the closely related benzyl-tetrahydroisoquinolines offer greater scope for the introduction of O-substituents. The total yield of capauridine was 1.2 gm.

The filtrate from the crystallization of capauridine on cooling and scratching yielded a copious crop of a second alkaloid. When once obtained crystalline this was only sparingly soluble in hot methanol from which it was recrystallized. After two such operations *capaurine* was obtained in very pale yellow, stout, irregular prisms, melting sharply at 164° C. In cold concentrated nitric acid it dissolves with the production of an intense cherry-red color passing into brown on heating. Cold concentrated sulphuric acid dissolves it to a colorless solution which, on heating, suddenly develops a very intense brown color which is changed only in intensity on dilution with water. Calcd. for  $C_{21}H_{27}O_5N$ : C, 67.56; H, 7.24; N, 3.67; 4 OMe, 33.24%. Found: C, 67.66; H, 6.70; N, 3.98; OMe, 33.15% (mean of duplicates).

The arguments against a formula with two hydrogen atoms fewer are the same as in the case of capauridine, although in both cases such a formula would agree equally well with the analyses.

Some more capaurine as well as a small quantity of capauridine was obtained from the fraction (EC), making the total yield of the former 21.4 gm.

#### *Methylation of Capaurine*

One gram of the base was dissolved in hot methanol and the rapidly cooled solution treated with 5 gm. of nitrosomethylurethane. A saturated methanolic solution of potassium hydroxide was cautiously added until an excess was present. The mixture was placed in the ice chest for six hours, then somewhat diluted with water and the methanol distilled on a steam bath. The insoluble pasty base was gathered on a glass rod and washed with water. It was then stirred up with aqueous potassium hydroxide containing a little methanol. The product, which rapidly became granular and crystalline, was filtered off and thoroughly washed with water. After drying it melted at 150-151° C. Recrystallization from hot methanol in which it is only sparingly soluble yielded stout, colorless prisms melting sharply at 152° C. Calcd. for  $C_{22}H_{29}O_5N$ : C, 68.22; H, 7.49; N, 3.62; 5 OMe, 40.05%. Found: C, 68.20; H, 7.11; N, 4.07; OMe, 40.55%.

#### *Methylation of Capauridine*

The procedure described for the methylation of capaurine was closely followed, except that owing to its sparing solubility the suspension of the fine crystals in methanol was employed. The quantities used were 0.3 gm. of the base and 2 gm. of the nitrosourethane. As before the crude base readily crystallized. It was recrystallized twice from hot methanol in which it is readily soluble. As thus obtained it consisted of colorless, stout needles melting sharply at 142° C. When admixed with an equal quantity of capaurine O-methyl ether the mixture began to sinter at 135° C. and melted completely at 140° C. The substance has not yet been analyzed.

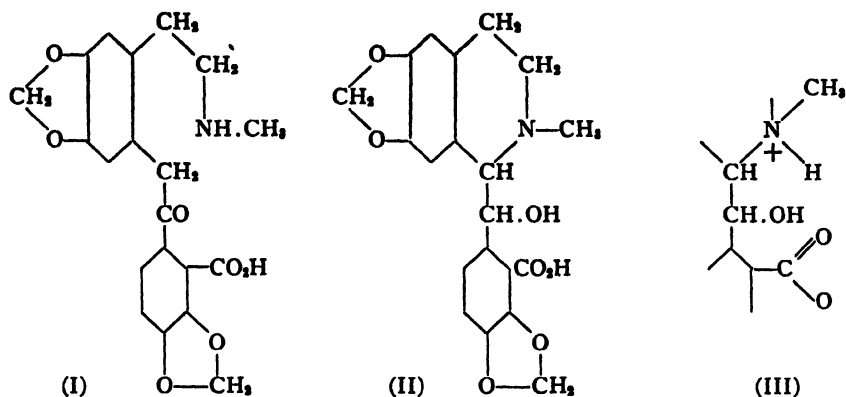
### *Isolation of Bicuculline and Bicucine*

The mother liquors from the isolation of the *L*-tetrahydropalmatine were extremely complex and this complexity was not greatly lessened in the fractions (EC) and (BCE). The combined mother liquors from which no more capaurine and capauridine could be obtained were exactly neutralized with concentrated hydrobromic acid, largely freed of solvent and repeatedly evaporated with chloroform. The thoroughly anhydrous solution deposited a small amount of gummy material which was removed by filtration through a layer of charcoal. The somewhat concentrated filtrate was treated with ethyl acetate until the incipient turbidity just disappeared on shaking and gentle warming. During the course of several days a colorless hydrobromide separated. This was cautiously washed with a mixture of methanol and ethyl acetate and the base regenerated by treating a warm aqueous solution with ammonia. The base which rapidly became granular was filtered off, washed, dried and recrystallized from chloroform-methanol. There was thus obtained 3.9 gm. of stout, colorless prisms, melting sharply at 177° C. The resolidified substance melted at 194-195° C. and this dual behavior was repeated when a specimen of bicuculline from *D. cucullaria* was admixed with it.

The mother liquor from the above-mentioned bicuculline hydrobromide was freed of organic solvents, dissolved in water, and the clarified solution fractionally precipitated with ammonia, the liberated bases being thoroughly extracted with ether at each stage. Of the five fractions, the first consisted almost exclusively of bicuculline, while the second and third yielded a little more (total 0.2 gm.). The remaining fractions could not be induced to crystallize. They were therefore combined, dissolved in dilute hydrochloric acid and basified with excess potassium hydroxide. The alkaline filtrate on saturation with carbon dioxide yielded a semicrystalline base which was filtered off, washed with water and dried. Extraction with chloroform and with cold methanol left 0.2 gm. of almost colorless crystals which melted at 209° C. This base was dissolved in cold concentrated ammonia solution and the clarified filtrate allowed to remain in a partial vacuum over concentrated sulphuric acid. In the course of 24 hr., fine colorless needles of *bicucine* melting either alone or admixed with an authentic specimen at 217° C. had separated.

### *The Constitution of Bicucine*

During the course of a further investigation of *D. cucullaria*, having as its object the isolation and characterization of the minor alkaloids, a considerable quantity of bicucine has become available and it has gradually become evident that it bears an exceptionally close relation to bicuculline. In the first place the two alkaloids have been invariably found together in the same plant and the possibility of their interconversion seemed a reasonable one. It has now been shown that hydrolysis with excess alkali is sufficient to convert bicuculline into bicucine, the latter being precipitated from the alkaline solution by a stream of carbon dioxide. Two formulas (I) and (II) are therefore possible,



The first was preferred for some time because it bears the same relation to bicuculline that nor-narceine bears to narcotine, although this change is brought about by a different method. Furthermore, a substance of formula (II) would be expected to form an anhydride with exceptional ease and yield the alkaloid bicuculline. It had been previously observed that oxidation with alkaline permanganate yields 3:4-methylene-dioxyphthalic acid, but direct evidence for the existence of the 4:5-methylene-dioxy grouping was not then available.

The reconversion of a substance of formula (I) into bicuculline is however quite inconceivable and the fact that, as now observed, boiling with dilute hydrochloric acid brings about an equilibrium transformation, definitely favors formula (II) in spite of its stability under other conditions. Furthermore, a solution of the base in either acid or alkali is optically active and formula (I) cannot accommodate this fact.

The stability of the  $\gamma$ -hydroxy acid complex is probably due to the fact that the alkaloid represents a comparatively stable dipole as shown in formula (III). The function of a strong acid in causing the formation of the lactone is therefore not only that of a catalyst in esterification, but more especially the removal of the charge from the carboxyl and the restoration of its normal properties.

The bicucine used in the following experiments was purified by solution in concentrated ammonia solution and removal of the ammonia from the filtered solution in a desiccator over sulphuric acid. The crystalline product was washed with cold water, dried and washed with chloroform. As thus obtained it melted at 215° to 217° C., but a specimen recrystallized twice from hot water and dried for 48 hr. at 60° C. melted with only slight decomposition at 222° C. This is the monohydrate,  $C_{30}H_{19}O_7N \cdot H_2O$ . In *N*/1 hydrochloric acid it showed  $[\alpha]_D^{25}, -145^\circ$  ( $c = 0.80$ ) 30 min. after solution and  $-100^\circ$  24 hr. later, and in *N*/10 potassium hydroxide it showed  $[\alpha]_D^{25}, -115.4^\circ$  ( $c = 3.20$ ). Obviously the mutarotation is due to the slow conversion of the base into bicuculline. Owing to the slight color in these solutions the above values are to be regarded as approximations only.

### *Conversion of Bicucine into Bicuculline*

Forty-four grams of bicucine was dissolved in boiling dilute hydrochloric acid and the solution heated on a steam bath for several hours. The solution was cooled somewhat and cautiously treated with aqueous potassium hydroxide until the mixture was distinctly alkaline to litmus. The precipitate was removed with a glass rod and thoroughly washed with boiling water. After drying it was dissolved in chloroform, the solution clarified with charcoal and the filtrate freed of solvent. The thick residual syrupy liquid was treated with hot methanol. Seeding with a crystal of bicuculline induced immediate crystallization. The yield of pure base melting at 177° C. (the resolidified base melting at 194-195° C.) was 15 gm.

The alkaline solution from which the bicuculline had been precipitated was made almost neutral with hydrochloric acid. The addition of aqueous sodium bicarbonate caused the rapid crystallization of bicucine: recovery, 11 gm. The process was repeated with some of the recovered base and yielded another lot of bicuculline. The optical rotation of a specimen of carefully purified bicuculline from *D. cucullaria* was determined in chloroform and showed  $[\alpha]_D^{25}, +130.5^\circ$  ( $c = 4$ ). Hydrastine in the same solvent is levorotatory ( $-67.8^\circ$ ) and it is not improbable that the two bases possess a different orientation of the asymmetric centres. In this connection, Prof. V. E. Henderson of Toronto University, in whose laboratory the pharmacology of bicuculline is being investigated, has found that it is 50-100 times as toxic as hydrastine. Since the physiological action is largely associated with the isoquinoline nucleus, the benzyl-lactone group serving largely as a modifier, slight changes in the latter would scarcely be expected to bring about such an increase in toxicity. Having in mind however the frequent inequality as to physiological activity of optical enantiomorphs, an explanation of the unusual toxicity of bicuculline is possible.

### *Oxidation of Bicucine with Permanganate*

A solution of bicucine in a large volume of dilute potassium hydroxide cooled with ice was treated with a saturated solution of potassium permanganate until the color was permanent for 15 min. The precipitated manganese dioxide was filtered off and the filtrate acidified and repeatedly extracted with ether. The residue from the extract was evaporated twice with alcoholic ethylamine and the solid distilled under greatly reduced pressure. The solid distillate after one recrystallization from ethanol yielded colorless fine needles of *N*-ethyl-3 : 4-methylenedioxy-phthalimide melting at 130° C. either alone or when admixed with a specimen prepared from bicuculline (3).

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# STUDIES ON THE APOIDEA OF WESTERN NOVA SCOTIA WITH SPECIAL REFERENCE TO VISITORS TO APPLE BLOOM<sup>1</sup>

By C. E. Atwood<sup>2</sup>

## Abstract

This paper describes the results of studies on the wild bees of Nova Scotia, which were carried out in connection with apple pollination investigations in the Annapolis-Cornwallis Valley, Nova Scotia.

The biology of the Apoidea in general is reviewed from the literature, and a list of bees taken on apple bloom is given. As the members of the genera *Halictus* and *Andrena* were found to be the most important native pollinators, the greater part of the paper is devoted to accounts of the habits and life histories of representative species.

The members of the genus *Andrena* were found to have a simple type, such as is generally found among solitary bees. The females provision the nest and then die; the larvae develop to the pupal stage in their underground cells, then emerge as adults the following season. All Nova Scotian species studied were one-generation forms.

The bees of the genus *Halictus* show a primitive social organization, more complex in some species than in others. The first brood consists of females only, which are apparently sterile and work at nest construction, the gathering of pollen, etc. They are followed later in the season by a brood of males and females; these females, after being fertilized, hibernate for the winter, while the males die in the fall. The hibernating habits of different species are described, and notes are given on some parasites andinquilines of the two genera.

## Introduction

The study of the wild bees of western Nova Scotia was undertaken in connection with an investigation of the pollination of the apple in this region. This project was sponsored by the Federal Department of Agriculture and was under the direction of Dr. W. H. Brittain. During the first two years statistical studies of the numbers present on bloom were made, and these showed the need of more detailed knowledge of the biology of the species concerned. It was immediately apparent that the bees of the genera *Halictus* and *Andrena* were the most important agents concerned except where hive bees were present, and therefore the greatest amount of attention has been given to these groups. In some cases bumble bees appeared to be of considerable importance, but their average number is too small for them to be considered a very large factor. Moreover their classification and life history are well known. Life history studies under field conditions were accordingly made with the genera *Halictus* and *Andrena*, especial attention being given to those species found to visit apple bloom. A classification of the groups has also been attempted.

## General Biology of the Apoidea

This group is the largest one among the Hymenoptera aculeata, having been estimated by Friese (4) to contain 20,000 species, the great majority occurring in the warmer parts of the north and south temperate zones. There is some difference of opinion as to the type from which they have evolved.

<sup>1</sup> Manuscript received August 23, 1933.

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Muller (6, 7), von Alten (1) and Friese (4) maintain that bees have evolved from the Fossores, probably by separate evolution from two or more distinct stocks. Roubaud (8), and Wheeler (9), who reviews the conclusions of previous writers on the subject, believe that the Sphecidae and Apidae (Apoidea) have both evolved from some earlier common Bethyloid stock, and that probably the vegetarian habits of bees may be a continuation of the plant-feeding habits of the Phytophaga or saw flies and their allies.

The great majority of bees are solitary, *i.e.*, one female makes a nest, provisions it, lays her eggs, and dies before the young emerge as adults. Their habits as a whole are "very monotonous," as Wheeler says, although there are individual differences for every species. The nests are made in wood or in the ground, or in crevices in stone. Many use the deserted holes of other bees, or of wasps, beetles and earthworms; Fabre and later writers mention several species which make use of empty snail-shells. Others construct cells from bits of leaf, clay, resin, down from plants, films of salivary secretion and other materials. Inside the cells a mass of pollen moistened with nectar is placed; this may be in the form of a smooth ball, or may be of a more or less fluid consistency and conform to the shape of the bottom of the cell. In this pollen an egg is laid; the entrance to the cell is then closed and the egg hatches and develops to the pupal stage in the cell. Many solitary bees build their homes in groups, and a few even use a common entrance, but there is apparently no approach to the social condition, except in the groups mentioned below. Typical genera of solitary bees are *Andrena*, *Melissodes*, *Ceratina*, *Megachile* and *Osmia*.

The typically social bees belong to the genera *Allodape* and *Halictus*, and to the subfamilies Bombinae, Meliponinae and Apinae of the Apidae. Only certain species of the first two groups are social, while all the species of the last three are. Wheeler (9) considers that the social habit has arisen separately in each of these groups, and that *Allodape*, although its social life is very primitive, is not in any sense ancestral to the higher forms. Brauns (2) has described the habits of this genus and states that they usually nest in pithy stems, as *Ceratina* does, but may occasionally be found in the ground. The female makes a nest or clears out an old one, then lays an egg. When this hatches she brings pollen to feed the larva, thus differing from the solitary bees which first provide the food, then lay the egg. When the first females emerge they help for a time to feed the young brood but eventually form colonies of their own. In the more primitive species, a large mass of pollen is brought to the larva at one time which suffices to feed it until it pupates, but in other species the provisioning is progressive, each load of pollen being divided among several larvae. In the former case also the eggs are very large and are laid at fairly long intervals, while in the latter they are small and several are laid almost at the same time.

There has been a great deal of controversy about the nesting habits of *Halictus*, and in fact various groups within the genus have decidedly different habits. The different types will be discussed under the treatment of that

genus but may be briefly indicated here. The nest is usually in the ground but may be in rotten wood, and is started in spring by one or more overwintered fertilized females and consists primarily of a narrow tunnel with cells excavated along it. These cells may be single, in groups, or, in two European species at least, in the form of a rude comb. The female kneads a mass of pollen moistened with nectar into a nearly round pellet and lays a single egg upon it. The grub hatches, devours the pollen, pupates and finally emerges by digging through the earthen plug which the mother places in the mouth of its cell. Some species of *Halictus* have only one generation; others have two, both containing males and females. Both these types are truly solitary. In yet another species the first eggs develop into sterile females which correspond to the workers of *Bremus*. These help the mother in the provisioning of the nest. She stays in the tunnel, guards the entrance and lays more eggs of which those laid last in the season produce some males and some females. The males die after fertilizing the young fall brood of females; the latter hibernate, and in the spring form a new nest or clean out the old one to receive their progeny. The mother dies at the end of the season along with the males.

The *Bremus* colony is usually started each spring by a single fertile queen, much as in some types of *Halictus*. The queen produces sterile female workers through the summer; males and females toward fall; the females hibernate after mating, while the males die. In some tropical species colonies are said to be perennial and new colonies are formed by swarming instead of by the dispersion of the fertile females in the fall, but in all species in the temperate zones the old queens die each fall.

The *Melipona* colony consists of a fertile female and several sterile female workers, her sisters, which have "swarmed" from the parent colony. The *Apis* colony also consists of a fertile queen and a number of sterile females or workers, but in this case it is the mother which heads the swarm from the old colony, taking with her some of her daughters and leaving the colony to a fertile daughter queen and a number of sterile daughter workers. Males are produced only at certain periods in both these types.

### List of Bees Taken on Apple Bloom

*Ceratina calcarata* Robt. (1 specimen)

*Megachile* sp. (1 specimen)

*Nomada* sp. (1 specimen)

*Sphecodes* sp. (1 specimen)

*Osmia* spp. (1 female, 3 males)

*Bremus ternarius* Say.

*B. terricola* Kirby.

*B. vagans* Smith

*B. fervidus* Fab.

*B. borealis* Kirby

*B. perplexus* Cres. (?)

*Psithyrus* sp.

*Halictus viridatus* Robt.

*H. foxii* Robt.

*H. macropinensis* Robt.

*H. pectoralis* Smith

*H. arcuatus* Robt.

*H. provancheri* D.T.

*H. rubicundus* (Christ.) Kby.

*H. coriaceus* Smith

*H. pilosus* Smith

*H. cressonii* Robt.

*H. zonulus* Sm.

*H. leucosonius* (Schrank.)

*Andrena milwaukeeensis* Graen.

*A. wilkella* Kirby

*A. bradleyi* Vier.

*A. thaspiis* Graen.

*A. vicina* Smith

*A. carlini* Ckll.

*A. crataegi* Robt.

*A. miranda* Sm. (*A. hippos* Robt.) (?)

*A. rugosa* Robt.

*A. mariae* var. *concolor* Robt. (?)

*A. lata* Vier.

*A. grandior* Ckll.

*A. ceanothi* Vier.

### Relative Abundance of Species

Exact data on the relative abundance of the various species of wild bees which visit apple are rather hard to obtain. The characters which separate many of the species are microscopic, so that an observer can determine the bees seen on the flowers only in a very rough way by deciding that they belong to one of two or three general groups. For example, it would be scarcely possible to separate *H. viridatus* Lov., *H. foxii* Robt., *H. cressonii* Robt., and possibly one or two other similar species, without a closer inspection than is usually obtained in the field. Even a count of species collected from bloom does not give a very definite idea of the numbers present, because of the fact that some are very easily captured while others are readily alarmed and difficult to net. However, from the combined results of four seasons' collecting and observation, a general idea has been gained of the numbers present belonging to the various groups.

It is quite apparent from even a short superficial observation that the small *Halicti* of the *viridatus* group mentioned above are more numerous than any other wild bees, provided the weather is suitable for general bee activity. Because of their small size (mostly less than 8 mm.) and rapid flight they usually pass unnoticed by the fruit growers, who do not realize the large part played by them in pollination throughout the orchards of the valley.

Probably second to these in numbers are some of the members of the genus *Andrena*. There is considerable local variation in the numbers of the different species present. In some areas *A. crataegi* Robt. is the most numerous; in others *A. wilkella* Kirby. *A. carlini* Ckll. and *A. vicina* Smith are often present in fair numbers, and from their large size and industrious habits are probably of great value. It is nearly always the female of these last two species which is taken on apple, whereas a large proportion of *crataegi* and *wilkella* are males. At one station *A. thaspii* Graen. was taken in considerable numbers, but as a rule this species appears to emerge too late to be of much value. Another group of *Halicti* which supplies a fair number of workers in orchards is composed of *H. rubicundus* (Christ.) Kby., *H. leucozonius* (Schrank.), *H. zonulus* Smith, and *H. coriaceus* Smith, while *H. arcuatus* Robt. is numerous in some localities. A bee which has been identified as *A. rugosa* Robt., and several closely allied species were present in the orchard at the Experimental Station, Kentville, in 1932, but were not found to be numerous at any of the other places where collecting was done. The other *Halicti* and *Andrenae* listed above may be regarded as occasional visitors only.

When present in appreciable numbers, the bumble bees of the genus *Bremus* undoubtedly are of considerable value, but the counts taken on apple bloom show that they are far outnumbered by the other wild bees. The numbers of bumble bees represented in the counts may not indicate their true value, because of the fact that they seem to prefer the tops of the trees, while most of the counts were taken within 10 ft. of the ground. Also they work at lower temperatures and in cloudy weather when other wild bees are not present. In an orchard at Blomidon during one season these insects were numerous

enough to be an important factor in pollination, but this is an exception to the general rule. The most numerous species seen in Nova Scotia orchards is *B. ternarius* Say, followed by *B. fervidus* Fab. and *B. vagans* Smith. Other species of *Bremus* and *Psithyrus* are occasional visitors only. The other bees given in the list must be regarded as largely accidental. Both *Osmia* and *Nomada* are present during apple bloom, but prefer blueberry, rhododendron, and other wild bloom.

Flies of many species, ranging in size from very small midges up to the large Syrphus flies, are frequent visitors at apple bloom. According to the counts taken of visitors to the blossoms they are much more numerous than bumblebees, but less numerous than other wild bees. However, it is very doubtful if the frequency of their visits can be taken as an index of their usefulness. Many of the flies recorded from apple do not come in contact with the stigmas at all because they simply alight on the petals and eat a small quantity of pollen, without entering the flower as the bee does. The flies, of course, have no interest in gathering pollen, they are simply eating a small quantity to satisfy immediate needs. Others, however, especially the Syrphidae and Bombyliidae alight on flowers and walk over the stamens and pistils much as the bees do. Although pollen-collecting apparatus, such as bees have, is absent, a fair number of loose grains adhere to the body hairs, and are transported from flower to flower. The counts on bloom show also that the number of flies present fluctuates in a very erratic manner, and it is apparent that they cannot be relied upon to effect adequate pollination in the absence of other agents.

### Notes on the Life Histories of Bees Taken on Apple Bloom

The following life history notes on some representative species of bees taken on apple bloom are compiled from observations made during the seasons 1929-1932. The method of study consisted of observations on the bees at work in the field, and statistical analyses of nest contents. In most cases individual nests were excavated and their contents noted, but in others the tunnels were so close together in the ground that it was impossible to follow single holes to their end or to ascertain their contents accurately. Therefore in some of the counts only the totals for an area excavated are given. The most extensive observations were on the genus *Halictus* as they persist throughout the summer, whereas each species of *Andrena* lasts for a few weeks only; moreover their nesting habits are comparatively simple and are much better known than those of the *Halicti*.

In 1931 some attempts were made to rear the *Halicti* in artificially constructed cells. None of the attempts were successful in rearing the insect from egg to adult, although larvae which had pupated emerged successfully. The chief difficulty was in controlling moisture conditions; the larvae were readily attacked by a mold when removed from the cells in which the egg had been laid, if kept moist; on the other hand the larvae rapidly succumbed to any condition approaching dryness.

None of the bees whose life history has been studied appear to confine themselves to any one family of plants in gathering pollen. Most of those found on apple may be taken at the same time on *Vaccinium*, *Rhododendron*, *Amelanchier*, *Prunus*, *Fragaria*, *Taraxacum*, etc., as well as on other plants which are in flower after the apple bloom has gone.

### 1. *Halictus arcuatus* Robt.

The nests of this bee were found in one place only. In this area, which occupied the upper slope of a steep pasture, there was a community consisting of hundreds of holes. The slope was composed of a loam with the following components:—sand, 69.4; silt, 21.8; clay, 7.8; volatile matter, 2.6–3.4%; and numerous small boulders. It was sparsely covered with grasses and weeds such as asters, goldenrod, thistles, wild carrot, and similar plants of hillside pastures. Nests were made either in the small areas of bare soil or among the roots of the grasses and weeds.

The entrances to the nests were usually very inconspicuous and difficult to discover. They were roughly oval in outline, but irregular, resembling the weathered burrow of an earthworm. Inside the entrance the tunnels were circular in cross section, very crooked, and more nearly on the horizontal plane than those of any other species studied. The cells in which the pollen was stored and the young reared were ovoid, joined to the main tunnel by a narrow neck, and finished inside with a smooth waterproof coating made of fine clay mixed with saliva. The pellets were larger in proportion to the size of the bee than those of the other species studied, and somewhat flattened instead of being spherical.

*H. arcuatus* begins its activities early in the spring. It is a common visitor to apple, wild cherry, etc., and, as early as May 18, holes were found containing fully formed pellets. Since the females do not hibernate in the summer holes, the nests found at this time must have been completely excavated since the bees began work. Therefore it probably emerges from hibernation in April or early May.

The eggs are white, translucent, narrowly elliptical, and slightly curved, so that they fit closely to the surface of the pollen pellet. When it hatches the larva feeds on the pollen pellet until it has consumed it, then pupates and finally emerges as an adult. Owing to the underground habit of the bee and the difficulty of rearing artificially, the exact length of the various stages could not be definitely ascertained. However, some notes made on the time at which different stages were found give a general picture of the life history.

The first larvae appear about the first week in June; the first pupae about June 20. The pupae seen at this time are all females and may be distinguished by their large size and stoutness. These females remain in the nest and bring in more pollen to provision cells. Whether or not they lay eggs has not been determined. An occasional male pupa may be found as early as July 15, but as a rule the greatest numbers begin to appear about two weeks later. Slightly later than this again the fall brood of females appears and by the end of August practically all pupae have emerged. About this time all the females

except the fall brood apparently die, while the latter, after mating, seek some other locality for hibernation. Males and females were never observed *in copula*—but mating probably takes place on flowers. The hibernating place of this species has not been discovered, but it is not on the site of the summer colony as careful digging there in the fall failed to show any signs of hibernating bees. From the fact that two females have been taken in one tunnel in the spring before larvae could possibly have developed, it would appear that two bees sometimes co-operate in digging the summer nest when they emerge in spring, and several may hibernate in the same hole.

Very few parasites were found in the holes of this species. In 1930 several newly hatched *Sphecodes* were found in one hole which was excavated, but none were taken from nests in 1931. A Conopid fly was very numerous in this region and dozens of them could usually be found nesting on grass leaves. They were often observed to dash at bees as they flew past and strike them in mid air, whereupon both would fall to the ground and struggle for a few seconds. The bees seemed much alarmed by these attacks, and as the Conopidae are known to lay eggs on other adult insects in this manner it was thought that the flies were probably parasitizing the bees (*vide* de Meijere, (5)). However, the great majority of the flies seen on the grass were males, and upon further observation they were observed to strike females in mid air in precisely the same way as those seen pursuing the bees. None of those seen attacking or striking bees were females, so the question is not definitely settled. It may be possible that the males mistake passing bees for female flies. The flies were, however, never observed to enter the nests, and no maggots were found.

Owing to the nature of the entrance, it was impossible to measure the number of holes in a given area, but instead an analysis of a given area was made in 1931 and 1932. In 1931 an area of 9 sq. ft. gave 109 pellets, 4 pupae, and 78 adults or 191 insects in all stages. Similar excavations in 1932 indicated a population of 198 per sq. yard. In the latter count many dead adults were found in the soil, probably the result of much wet cold weather in the spring.

This species apparently has two forms of female, the spring form being larger and more robust, with more pronounced abdominal fasciae and a shorter dorsal space to the propodeum. The smaller form has been taken frequently on flowers during the summer and was also found living in nests with the larger females. The question is considered more fully in the paper on the taxonomy of this genus.

## 2. *Halictus leucozonius* Schrank.

This member of the genus nests in a great variety of situations and is very plentiful and very widely distributed in the Annapolis Valley region. The favorite habitat appears to be a somewhat flat field or pasture covered with short thin grass, the character of the soil apparently not being an important factor, as long as it is firm and not composed of coarse gravel or pure sand. Many large communities were found in pastures of this character, but nests

were also commonly found along roads and ditches, between rows of trees in orchards, on open clay or loam banks and the drier parts of open diked lands.

The entrance to the nest of this species is  $\frac{1}{4}$  to  $\frac{1}{2}$  in. in diameter at the entrance, slightly wider inside, and usually enters the ground perpendicularly. As a rule it is straight for the first two or three inches, and sometimes for the whole length, although there is usually a slight crook somewhere in the tunnel. Around the entrance there is always a low mound of earth, except on a slope where the earth falls downward as it is brought out. This mound is about  $1\frac{1}{2}$  in. or more in diameter, and is hollowed at the top so as to form a crater about the entrance. The tunnel and cells are lined with the usual waterproof lining. The latter are about  $\frac{1}{2}$  in. long, ovoid in shape, and joined to the main tunnel by a neck slightly longer than the length of the cell. This neck is generally filled with earth after the cell has been provisioned.

The females of this species do not begin nest building much before June 1. In some cases two females must co-operate in digging, because more than one adult was occasionally seen in a hole much earlier than the appearance of the pupae in the cells. There are also great differences between the times of nest building of the females. For instance, in one location with a heavy clay soil, many newly made nests were found on July 21, while, a few feet away, other holes of the same species contained larvae and pupae.

During the provisioning of the cells the adults have a curious habit of digging out some earth from down in the tunnel, probably from the cells, pushing it up as far as the mouth of the hole and leaving it there where it forms a plug slightly below the level of the top of the cone around the entrance. This plugging of the nests was found to take place in many nests between 11 a.m. and noon. The plug was sometimes removed in the afternoon, sometimes left until next morning. The females do not usually stand guard at the mouth of the tunnel, so this plug may act as a sort of door to keep out intruders. It must be quite effective, because very few parasitized cells have been found while excavating holes of this species.

It would appear from the counts of larvae, pupae, and adults from excavated holes that there is no great emergence of females before the appearance of the males, because males were found within a few days of the discovery of the first pupae. This would indicate that the only females produced are those which hibernate during the winter, although this is not definitely proved.

The habits of the males of this species are similar to those of *H. rubicundus* (Christ.) and *H. provancheri* D.T.; that is, they wait in holes and crevices and around sunny open spots for the appearance of females. They also are taken on goldenrod, fall dandelion, and other flowers in large numbers, but mating probably takes place in the former situations, although it has not been observed.

Apparently the females do not hibernate in the summer nesting site, as such areas have been searched without finding any dormant bees. The males persist until late in the fall; some were noted on October 10 after rather severe frosts,

Attempts were made to estimate the numbers present in some of the more densely populated areas. Counts taken on measured areas in 1931 gave an average of six to seven holes, or between 25 and 30 bees per square foot. This would perhaps be a rather large estimate for a pasture or field with a small population, but there were large areas near Grand Pré, Horton, Welsford, Port Williams, and other locations where this figure would not be excessive.

*H. zonulus* Sm., a closely related but rarer species in Nova Scotia, has very similar habits, to judge from the few nests found. It apparently prefers somewhat flatter and more moist locations for its nest.

### 3. *Halictus rubicundus* (Christ.) Kby.

This species is the largest one of the genus found in Nova Scotia, and is frequently seen on apple bloom. It nests in various types of soil, the holes usually being very scattered, but in one location an extensive colony was found nesting on the inner face of a dyke. This dyke was composed of sods cut from the silt brought down by the river, and held together by the roots of sedges and grasses. In other localities the holes were chiefly found in rather dry firm soil, sparsely covered with grasses.

The hole is usually  $\frac{1}{4}$  in. or more in diameter at the mouth, slightly larger inside, nearly perpendicular to the surface, and usually straight or at most with a slight turn about halfway down, although some are more crooked. The holes seldom reach a depth of more than 8 in. They are usually simple, but occasionally a branched one was found, which had apparently been made by two females using a common entrance. Around the top of the nest a small cone of loose earth is usually found, unless the hole is situated on a slope.

The cells are of the usual type, oval,  $\frac{1}{2}$  in. or more long, with a smooth firm lining, and joined to the tunnel by a narrow neck.

*H. rubicundus* begins work sometime in early May, at least in the lighter soils, as completed cells were found as early as May 20. The first summer females emerge as a rule about the middle of July or a little earlier, the males appearing some two weeks or more later. Other females and males continue to emerge-until well on in August. The males wait in holes and on open sunny spots for the females to appear and mating takes place on the ground; they are also, however, found on various flowers drinking nectar.

The fertilized females may dig new holes in which to hibernate, or may merely dig a summer nest a few inches deeper, and partly fill the upper section with loose earth. Hibernation appears to take place at a depth of about 12 in. below the surface. It begins about August 20, and by September 1 most of the females have disappeared, although the males remain until killed by frosts.

A few cells were found which contained the maggots of a parasitic fly, but no other enemies of the bee have been discovered in this region.



#### 4. *Halictus provancheri* D. T.

No nests of this bee had been seen before the spring of 1931, although the holes made by two hibernating females had been found the previous summer. The preferred habitat of the species seems to be a well-drained soil with a covering of short grass or other low vegetation. In each nesting site found, except one, the soil was light and rather loose; the exception being the dyke described as a nesting site of *H. rubicundus*, which had been made from sods of a brackish marsh. A covering of short grass or other vegetation appeared to be an essential feature of the nesting site, as every hole found was in such a situation.

The nests of this bee were more difficult to find than those of any other *Halictus* studied. The entrances were always practically invisible until a bee was seen to enter one, and even then the debris on the surface usually fell together over the hole as soon as the bee had entered. Most tunnels opened either at the base of some plant or else actually among the stems of a grass clump. Owing to the light dry nature of the soil in which the nests were made it was impossible to excavate them in such a way as to observe the structure very carefully, but they were apparently nearly vertical after the first inch or so, the upper part coming in at an acute angle to the surface and then turning several times before becoming vertical. The cells were of the general *Halictus* type, but not as numerous as those made by other species; five was the greatest number of inhabitants taken from a single hole.

The females had a most annoying habit of hovering around and lighting on grass for an hour or more before entering the hole, even when carrying a load of pollen. In one case, some of these females were watched continuously from 1 p.m. until 4.30 p.m. and had not entered the hole at that time. This hesitation to enter the hole was repeatedly observed, but is rather hard to understand, because it must lead to a great waste of time in pollen gathering. A large number of a species of parasitic fly were observed in and around the nests of this species and they may have had a disturbing influence on the activities of the bee. Many cells were found which contained dipterous larvae, while puparia were often found in the soil near the tunnel, the maggots apparently leaving the cell to pupate. This heavy infestation of parasites may be responsible in part for the comparative rarity of *H. provancheri* in this part of Nova Scotia.

The earliest date on which holes of this species were excavated was June 8. At this time the hole was still in the course of construction but some pollen had been brought in. Young larvae were found some two weeks after this date. Males began to appear early in July, but were most abundant during August, when they were very numerous around the dyke mentioned above. It would appear that very few if any females could emerge before the males begin to appear, so the species must be like *H. leucosoni* (Schrank.), having but one brood per season. The collecting of pollen, however, must be kept up over a considerable period as pupae were found as late as July 31.

Males were taken on various flowers, but were most numerous around open sunny spots near nesting sites, and mating was observed to take place in such localities. It was also, however, observed on flowers in 1932. The females begin to hibernate soon after the middle of August. They dig new holes for the purpose, in light sandy loam, partly filling the hole with loose earth.

#### 5. *H. viridatus* Lov.

This bee is more numerous and more widely distributed than any other species of *Halictus* taken in the apple growing districts, and in spite of its small size it is of great value in orchards, especially as it is about the first of the genus to appear in the spring. Its life history is more complicated and interesting than that of any other species studied, and shows a near approach to the social habit.

Bees of this group have been found nesting in so many types of soil that it is very difficult to state what is, or is not, a favorable habitat. Apparently any firm well-drained soil except pure sand or coarse gravel can be used. A slope is sometimes preferred, but is not at all essential in a nesting site; in some places the uncultivated strip between rows of orchard trees was a favored spot. A bare soil or one sparsely covered with grass is preferred; a few holes were found in thick grass but they were the exception. The soil from two favored sites was analyzed and proved to have the composition shown in Table I.

TABLE I  
COMPOSITION OF SOIL

Constituent	Site No. 1	Site No. 2
Sand, %	80.0	79.3
Silt, %	14.8	16.3
Clay, %	4.4	3.7
Volatile matter, %	2.6-3.4	2.6-3.4

South, southeast or southwest slopes were preferred, but nests were found on slopes of all directions, including north. In very favored localities the nests were often so close together as to suggest that the bees were somewhat colonial or rather gregarious in habit, but under average conditions the nests were quite isolated, so the grouping of holes was probably due to the attraction of the favorable site rather than to any preference for the company of other members of the species.

The openings of the tunnels are very regular and smooth, and circular in outline; as a rule they enter the ground nearly at right angles to the surface. Usually no particles of soil were left around the hole, but in some cases, especially where the holes were constructed among grass stems, small cones remained about the excavation, composed of the material removed from the tunnel and cells. Many of the holes were found on smooth surfaces, with no attempt at concealment, but occasionally they were under small leaves or grass blades slightly raised from the ground, or in the angles around projecting lumps of soil, small rocks, etc. In one case a hole was found in an area which was completely covered by a leaf of a young *Hieracium* plant;

the bee had cut a perfectly circular hole through the leaf directly over the entrance of the tunnel. Inside the entrance the tunnels of this group were by far the most complicated of those of any of the bees studied. The mouth is made of a size to fit the head of the bee so that when a female stands just inside the entrance the hole is effectually blocked. Immediately inside this entrance the hole is enlarged to two or three times the diameter of the mouth forming a chamber in which two bees may pass. The chamber is about the shape of an egg glass, with the entrance at a point corresponding to the bottom of the bowl. It narrows abruptly at its inner part to form a constricted passage of the same shape and size as the outer entrance. Beyond this is a second enlarged chamber; a third and fourth each with its round narrow mouth may be present. Inside the last chamber the main tunnel is slightly larger than the entrance, still smooth and circular in section, and lined with the fine clay and saliva mixture which most *Halicti* use.

The majority of holes examined consisted of a single winding tunnel 4 to 8 in., or more, in total length but seldom reaching a vertical depth of more than 6 in., below the surface. They were usually very crooked, winding up, down, or sideways but usually with a straight portion about 3 in. long immediately inside the area taken up by the entrance chambers; this was generally followed by a sharp curve, then various windings were found in the region occupied by the cells. However, not all tunnels were of this simple type, as branched ones were quite frequently observed. The branching in many cases took place just inside the entrance; sometimes it even opened off the first or second chamber. In other holes it took place below the straight portion mentioned above. In the first type, where the tunnel branched near the entrance, each branch often had secondary ones near the bottom, so that they were like two distinct branching tunnels with a common entrance. In some cases it appeared that there was a connection between two such branches near the bottom as well as at the entrance, but the cells of branched tunnels were always placed so closely together that it was hard to discover their exact relations to the main tunnel.

The holes of this group are very populous. It was quite common to find 15, 20, or more, inhabitants in a single tunnel. Only rarely were more than a dozen found in the tunnels of any other species. This may be partly due to the fact that many holes, even in spring, contain more than one female. Apparently when they come out of hibernation, a few of the sisters may scatter and find new homes, but, in many cases, two or more remain in the tunnel in which they passed the winter, dig it out again, reline the cells and work together in storing pollen and rearing young. A very good example of a hole of this kind was seen at Blomidon on June 5, 1931. A bee carrying pollen was seen to alight near a hole which was filled by the head of another female. The guard withdrew into the hole and the pollen-covered bee entered. The hole was then excavated and was found to have two branches which separated just inside the entrance, and to contain four adults, nine pellets with eggs, and two young larvae. It was so early in the season that all these

adults must have been overwintered females; it would have been interesting to have marked them to see if they took turns at standing guard. In any case their behavior is quite different from the species described by Fabre, which do not guard their burrows in the spring but only after the first pupae have emerged as adults.

In spite of the vigilant guard kept by the *Halicti* at the mouth of the tunnel, enemies enter, and many cells inhabited by inquilines were found while excavating the nests of these bees. The most common were the maggots of one of the Diptera, which has not been secured in the adult stage. Many Meloid beetle larvae in the triungulin stage, and one scarabaciform larva, were found. Occasionally an adult *Sphecodes* was found in a nest, but it may be mentioned here that on one occasion a *Sphecodes* was seen vigorously excavating a hole of its own, so it would appear that these females are not always socially parasitic. The guard at the entrance could not keep out the small beetle larvae which enter by clinging to the bee as she enters the nest, but the flies and the inquiline bees must enter when no sentinel is present. Parasitic Strepsiptera were also found more frequently on bees of this group than on any other species of *Halictus*, and many were infested with ticks as well. In 1931 a long period of cold wet weather in June killed many adults in the ground and made a decided gap in the collection of pollen. No other serious causes of mortality were noted.

The first males seen in the holes were found on July 13 and from that time on they were plentiful on flowers, showing that this was the beginning of the regular brood of males. Some of these may go back to the holes after emergence but as brood rearing continues long after this date, it is probable that they do not do so to any extent. None were ever noticed entering holes or hovering around them as some of the males of other species do. On the other hand, after rainstorms they were often found on flowers or leaves, stiff with cold and wet. When warmed up by the sun or in the hand they again became active. Some of them live until very late in the autumn; on October 3 several were collected on *Leontodon*, *Aster* and other flowers, together with females of the same species, and on October 6 an adult male, apparently just emerged, was taken from a hole together with two larvae. This last fact shows that brood rearing continues until late in the fall. In fact on October 3 many females were still taking pollen into their holes but this must have been due to the instinct of the insect to keep working as long as flowers are available and the temperature is not too low. Certainly pellets made at this time would not serve the purpose of rearing other bees.

The females apparently begin to construct hibernating quarters early in September, either by deepening the hole in which they were reared or by digging a new hole. At this time small cones of earth appear about the entrance of every hole. However they continue to go in and out of the hole and to visit flowers during fine warm weather until early October at least. As a rule many females hibernate together in the same hole. Males and females of this group were never seen *in copula*, but it is probable that mating takes

place on flowers, as the males do not have the habit of hovering about holes and nesting sites as do those of *H. leucozonius* or *H. rubicundus*. This species appears to have very little gregarious tendency, and holes were usually scattered all over any favorable spot. In some cases a particularly favored bank was found with a population of three to six holes per square foot over a small area, but these areas were always small.

#### 6. *Andrena carlini* Ckll.

This bee seems to be partly colonial in habit; in some cases scattered holes were found, in others some dozens nested close together. They are, like all of the genus, entirely solitary, each female digging a hole, provisioning it, and laying an egg in each cell. There is only one generation per year, the females appearing early in spring and dying off about the middle of June or a little later.

The tunnels are made in light soil, often with gravel and small boulders in it, and usually among grasses or other vegetation. They are about  $\frac{1}{2}$  in. in diameter, and often penetrate to a foot or more in depth, with many turns which make the total lengths much greater than this. The entrance to the hole is usually left open, but frequently becomes partly stopped by loose earth falling back from the rough mound left around the hole. The cells are at a distance of an inch or so from the main tunnel, and are provisioned with a mass of pollen and nectar. The connection between the tunnel and the cell is completely and firmly blocked with soil after the provisioning is done, making it difficult to tell just how many cells are present. The pollen mass is more nearly liquid than those of the *Halicti*. The cells were lined much as in that genus, but the tunnels seemed to be more roughly finished. The places where this bee nests are visited constantly by a species of *Nomada*, which undoubtedly is parasitic upon the *Andrena*. In some years cold wet weather also kills many of the adult females in the ground.

#### 7. *Andrena wilkella* Kirby

Only a few nests of this species have been found, but they are apparently of the ordinary *Andrena* pattern. The bee appears in greatest numbers in areas having a heavy soil, and most of the nests found were in such localities. The character of the nest was quite different in different types of soil. In a light sandy loam a hole was followed to a depth of 18 in. while in clay the hole was seldom more than 6 or 8 in. The total length was always greater than the depth, because the holes of this species are very crooked. The entrance was always difficult to locate because it was invariably among dense low grasses, lichens, or other cover. A small quantity of earth was found about some holes; others had none. The cells are finished with the usual coating of fine clay, but the tunnels are not. After the cells have been provisioned with a pellet of pollen and nectar, the connection to the main tunnel is firmly packed with earth and a new cell made in another place. The young remain in this cell until they emerge in the following spring as adults.

Males of this species emerge considerably before the females and are often very numerous about apple trees and various shrubs, often when there are no flowers present at all. The females persist until long after apple bloom and have been seen as late as August 1 on clover. In England, the species is said to nest in large colonies, but all the holes found in Nova Scotia were scattered, although colonies may have been present in the district.

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# TELIOspore DEVELOPMENT IN THE PUCCINIASTREAE<sup>1</sup>

By S. M. Pady<sup>2</sup>

## Abstract

The genera that constitute the Pucciniastreae display a wide variation in the type of teliospore produced, as well as in the time and place of production. From the standpoint of development, however, there is a general situation that is common. In all genera primordial cells are formed from enlarged hyphal cells of the mycelium. These give rise to teliospore initials which are in the epidermal cells in *Calyptospora*, *Milesia*, *Hyalopsora* and *Thecopsora*, and are subepidermal in the other genera. These initials divide to form the mature teliospores, which are thick or thin walled, and few to many-celled. In all cases the teliospore is the product of a single primordial cell.

The teliospores of *Calyptospora goeppertiana* are formed from a perennial mycelium, which causes a witches' broom and hypertrophied stems on species of *Vaccinium*. The mycelium gives rise to primordial cells in the cortex just below the epidermis. Each primordial cell pierces the host wall above and the contents pass in to form the initial, which by growth and division becomes the teliospore. The mature teliospores are one- to four-celled, with a thickened, dark brown wall. Development is not simultaneous, but progressive, and the teliospores are first formed in the basal parts, moving slowly upward until every cell of the hypertrophied portion of the stem is completely filled. In four species of *Milesia* the method of development is similar. The spores, however, are thin walled, and are formed in the epidermal cells of the overwintered fronds of their fern hosts. *Thecopsora vacciniorum* is similar to *Milesia* in many respects. The teliospores are intra-epidermal, thin walled and multicellular. In *Pucciniastrum* the teliospores are subepidermal, and arise from primordial cells, as in *Calyptospora*, *Milesia* and *Thecopsora*. The teliospore initials are closely packed, and the mature spores may form extended crusts. The simplest type of development is found in *Uredinopsis*, which is generally considered to be the most primitive of the fern rusts. Primordial cells are formed in the same way as in the other genera. These round up to form the initials, and cross walls are laid down to give the mature spores.

From these studies two possible lines of development are suggested, both beginning with *Uredinopsis*. One line would lead through the intra-epidermal forms, as *Milesia*, *Calyptospora*, etc., and the other through the subepidermal genera, as *Pucciniastrum* and *Melampsoridium*.

## Introduction

The Pucciniastreae constitute a subtribe of the Melampsoraceae, and are characterized by indehiscent telia and aecia of the peridermal type. The teliospores are intra-epidermal or subepidermal, and show a wide variation in time and place of development. In *Calyptospora*, *Milesia*, *Hyalopsora*, *Thecopsora* and *Melampsorella* the teliospores are formed in the epidermal cells, while in *Uredinopsis*, *Pucciniastrum* and *Melampsoridium* they are subepidermal. The general situation is shown in Table I.

From Table I it will be seen that there are considerable differences not only in the time of teliospore formation, but also in the position and in the type of spore produced. Little, however, has been known of the manner in which the teliospores develop in any of these genera. As Arthur (2, p. 149) has pointed

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TABLE I  
TELIOspore FORMATION

Rust genus	Time	Place	Type of spore
<i>Calyptospora</i>	During the growing season	In epidermis of the stems	1-4 celled, thick walled
<i>Milesia</i>	Following spring*	In epidermis of overwintered fronds	Multicellular, thin walled
<i>Hyalospora</i>	Early spring	In epidermis of young fronds	Multicellular, thin walled
<i>Thecopsora</i>	At end of the growing season	In epidermis of season's leaves	Multicellular, thin walled
<i>Uredinopsis</i>	At end of the growing season	In mesophyll below epidermis	1-4 celled, or many celled, thin walled
<i>Melampsorium</i>	At end of the growing season	Subepidermal	Single celled, thin walled
<i>Pucciniastrum</i>	At end of the growing season	Subepidermal	1-4 celled, thick walled

\*In one species teliospores may also be formed at the end of the current season.

out: "The development of the telial primordium in such genera as *Coleosporium*, *Melampsora*, *Melampsoropsis*, *Melampsorella*, *Uredinopsis*, *Milesia*, *Hyalospora*, *Pucciniastrum*, in which the teliospores are sessile and arise under the cuticle or within or beneath the epidermis, has received little study." The following investigations were undertaken with the object of studying the method of teliospore development, especially in the genera in which the teliospores are intra-epidermal.

The species of Pucciniastreae that have been studied are parasitic on ferns and flowering plants, three genera, *Uredinopsis*, *Milesia* and *Hyalospora*, being found on Pteridophytes, the remainder on Angiosperms. It might be noted here that with the exception of *Thecopsora vacciniarum*, the aecial host for all these species in eastern North America is *Abies balsamea*. The telial hosts are shown in Table II.

TABLE II  
TELIAL HOSTS FOR SPECIES OF PUCCINIASTREAE

Rust species	Telial host
<i>Calyptospora goeppertiana</i> Kuhn	<i>Vaccinium pennsylvanicum</i> Lam. and <i>V. canadense</i> Kalm.
<i>Milesia marginalis</i> Faull and Watson	<i>Thelypteris marginalis</i> (L.) Nieuwl.
<i>Milesia polypodophila</i> (Bell) Faull	<i>Polypodium virginianum</i> L.
<i>Milesia intermedia</i> Faull	<i>Thelypteris spinulosa</i> var. <i>intermedia</i> (Muhl.) Weath.
<i>Milesia fructuosa</i> Faull	<i>Thelypteris spinulosa</i> var. <i>americana</i> (Fisch.) Weath.
<i>Thecopsora vacciniarum</i> (D.C.) Karst.	<i>Vaccinium pennsylvanicum</i> Lam. and <i>V. canadense</i> Kalm.
<i>Melampsorium betulinum</i> (Pers.) Kleb.	<i>Betula pumila</i> L. (?)
<i>Uredinopsis phegopteridis</i> Arth.	<i>Thelypteris dryopteris</i> (L.) Slosson
<i>Uredinopsis struthiopteridis</i> Storm.	<i>Pteris nodulosa</i> (Michx.) Nieuwl.
<i>Pucciniastrum abielt-chamaenerii</i> Kleb.	<i>Epilobium angustifolium</i> L.
<i>Pucciniastrum epilobii</i> (Pers.) Otth.	<i>Epilobium adenocaulon</i> Haussk.
<i>Hyalospora aspidiotus</i> (Pk.) Magn.	<i>Thelypteris dryopteris</i> (L.) Slosson



## Material and Methods

All the rusts described in this paper are found in abundance in the Temagami Forest Reserve in northern Ontario. The field laboratory of the Department of Botany, University of Toronto, is ideally situated in this region, and was used as a base for this work during the summers of 1930, 1931 and 1932. When material was brought into the laboratory it was placed in a moist chamber, and if freehand sections revealed the presence of desirable stages small portions were removed and placed in various fixatives. In a number of cases fixations were made in the field, especially where the particular collecting ground was located at some distance from the laboratory. Of the many fixatives that were tried, Flemming's weak has proved to be the best, although formalin-acetic-alcohol was successfully used in certain cases. The latter fixative is advantageous in that it also acts as a preservative, and material may be left in it for an indefinite period. Flemming's triple stain gave the best results and was used throughout. Heidenhain's hematoxylin was occasionally used to supplement the triple stain, but on the whole was rather unsatisfactory.

Freehand sections were employed with considerable success throughout these investigations. They not only served to reveal the presence of desirable stages in preliminary examination, but also proved to be valuable for comparison with stained microtome sections. Many of the drawings included in this paper have been made from freehand sections. Excellent results were obtained from fresh material following fixation in 95% alcohol, or in formalin-acetic-alcohol. Large quantities of selected material were placed in the latter where they were available for sectioning at any time. Freehand sections of preserved material may show an extraordinary amount of detail, especially when mounted in lactophenol which contains a small amount of cotton blue, or some other stain.

## Species Investigated

### 1. *Calypsotheca*

The genus *Calypsotheca* was established by Kuhn (14) in 1869, with *C. goeppertiana* as the type species. The genus has remained monotypic though the combination *C. columnaris* (Alb. and Schw.) Kuhn is in more common use in America. Spermatogonia and aecia were not known at the time, and the description was based entirely on the character of the teliospores, which were found to fill the epidermal cells of the stems of the host. Kuhn described the teliospores as irregular, ellipsoid, prismatic, obtuse above, rounded below, subfuscous, paler below; divided twice, or sometimes several times by cross walls. Mycelium was found to be distributed between the cells of the cortical tissue, causing an abnormal enlargement of the same. As a result, the stems of *Vaccinium vitis-idaea* became thickened and sponge-like all round, although enlargement was occasionally found only on one side.

Hartig (11) in addition to describing the hypertrophied stems, carried out infection experiments on *V. vitis-idaea*, and studied the development of the teliospores. Aeciospore infection gave rise to mycelium which established

itself in the cortex. The following year the new shoots were changed into the hypertrophied form. The mycelium grew into the new shoots, and caused an enlargement of the cortical cells, and this action was continued as long as the cells remained young. At the tip of the shoot, the cells were mature and thus were not stimulated to greater size. The intercellular hyphae derived their food from the parenchyma cells by means of haustoria. "The spores originate from processes of the mycelial hyphae which bore their way into the epidermal cells, and well up inside to form spherical sacs. The cells thus entered turn brown and are filled with four to eight cells produced from the sac-like process of the mycelium. From each cell of this kind a four-celled teleutospore is formed, and hibernates *in situ*" (translation). The infected stems became swollen to the size of a quill and were very conspicuous. This thickened spongy part of the stem was at first whitish or somewhat rosy in color, changing later to a brown or blackish brown color. Hartig's description was accompanied by three figures. The first showed a typical branch from a witches' broom with well-marked hypertrophied stems, the second dealt with the club-shaped bodies below the epidermal cells and the perforation of the cell wall, while in the third figure the teliospores, which have filled the epidermal cells, were shown to be germinating. Nuclear details were lacking in all figures.

Tubeuf (21) reviewed Hartig's work and added a more complete description of the hypertrophied twigs. "The mycelium hibernates in the cortical tissues, growing intercellularly and sending haustoria into the host cells. As a result of its presence growth is much accelerated, and a marked thickening of the attacked twigs frequently occurs; intercellular spaces become enlarged and the contents of all cortical cells, except those of the epidermis, take on a rose-red color, though they afterwards turn brown" (translation). Five figures accompanied the description, three of which were the figures of Hartig, described above.

Grove (10) has given an account of the infected stems, which he found to be swollen and clear pink at first, later becoming brown. The teliospores were described as caulicolous, developed in the epidermal cells, densely-crowded, prismatic, mostly divided by crossed longitudinal walls into four cells, up to  $48\mu$  high, epispore yellowish-brown, smooth, thickened (up to  $3\mu$ ) at the summit, with a germ pore at the upper and inner corner of each cell. The teliospores were formed in the stems of *Vaccinium vitis-idaea* during July and August in England and in Wales.

Sydow (20) in his *Monographia Uredinearum*, has given an excellent review of the earlier work. His description of the teliospores was also very complete.

The purpose of this investigation was to study the development of these intradermal teliospores. What is the nature of the sac-like bodies described by Hartig? How many are formed per epidermal cell? How many teliospores are developed from one sac-like body? These are some of the questions this investigation has sought to answer.

*Calyptospora goeppertiana* is found in abundance in the Temagami region on *Vaccinium pennsylvanicum* and *V. canadense*. The fungus stimulates the host, resulting in excessive branching, and this gives the affected plant its witches' broom appearance, which is accentuated by the marked hypertrophy of the stems. The infected branches of the previous year are greatly hypertrophied, being three to four times the diameter of a normal branch, dark brown in color, corky in consistency and quite dead. In the spring a large number of new shoots appear from the base of these dead branches, and these are soon invaded by the perennial mycelium. The young infected shoots may readily be distinguished from normal shoots by their slightly larger size, pale green color, and soft fleshy appearance.

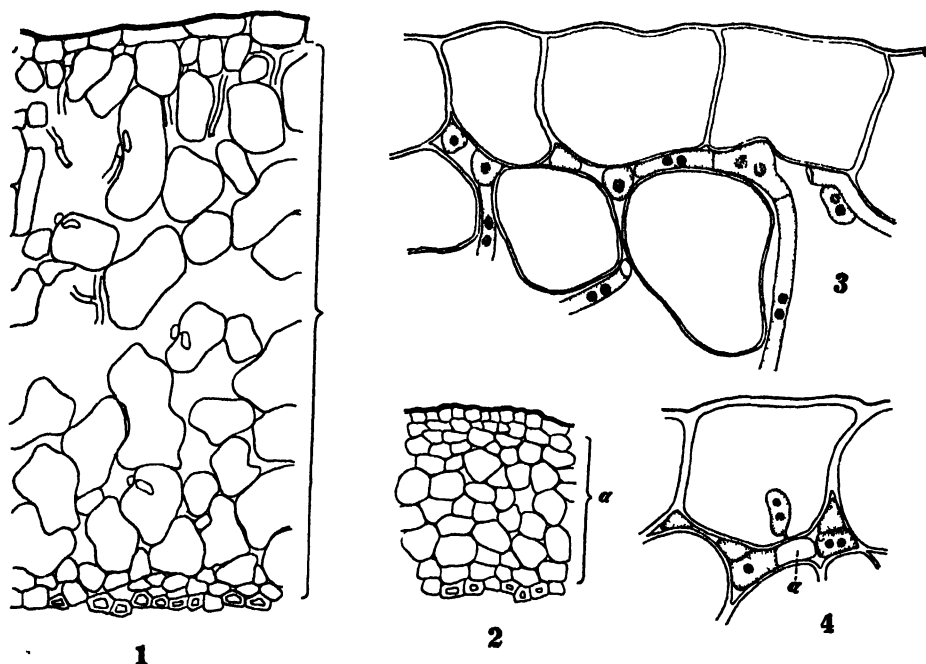


FIG. 1-4. *Calyptospora goeppertiana*. FIG. 1. Cross section of hypertrophied stem showing portion of cortex (a).  $\times 120$ . FIG. 2. Cross section of normal stem with only cortex (a) shown.  $\times 120$ . FIG. 3. Early stage. Mycelium aggregating below epidermis.  $\times 750$ . FIG. 4. Another view of the same stage. Note the haustorium and haustorial mother cell (a).  $\times 750$ .

The infected shoots continue their growth and reach a considerable length. The hypertrophied areas become more and more prominent, increasing in length as the shoot elongates, and also increasing in diameter until they reach a size three to four times that of the normal shoots. This is illustrated in Figs. 1 and 2. Fig. 1 shows a cross section of an infected stem, the cortex (a) only being drawn. Fig. 2 shows a corresponding section through the cortex (a) of a normal uninfected stem. It will be observed that the cortex in Fig. 1 is more than three times greater in thickness than the cortex of Fig. 2.

The mycelium grows rapidly through the intercellular spaces of the cortex and a section through the stem at this stage reveals the presence of mycelium in the large intercellular spaces, and abundant haustoria in the cells. The next change that may be observed is that the hyphae appear to grow outward, and become aggregated just below the cells of the epidermis (Fig. 3). Haustoria are found not only in the cortical cells, but also in the epidermis (Fig. 4). The haustorium in this figure has been formed from the haustorial mother cell (*a*) which is about the same size as the adjacent hyphal cells massed in the intercellular spaces just below the epidermal cells. The hyphal cells that lie in close contact with the epidermis now commence to increase very rapidly in size, and this is apparently the first step in the formation of the teliospores. These enlarged hyphal cells become the primordial cells, and this stage will be referred to as the primordial stage.

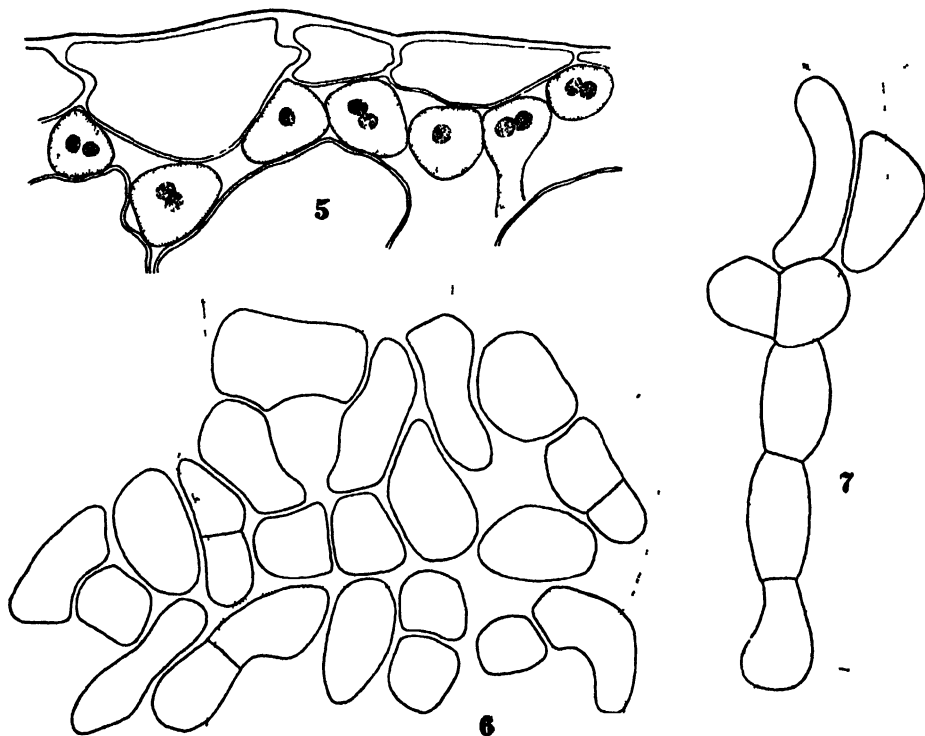


FIG. 5-7. *Calyptospora goeppertiana*. FIG. 5. Young primordial cells.  $\times 750$ . FIG. 6. Surface view of primordial cells. Walls of epidermal cell indicated by dotted lines.  $\times 840$ . FIG. 7. Young primordial cells at the junction of two epidermal cells.  $\times 840$ .

The young primordial cells form a loosely arranged layer just below the epidermis (Fig. 5). They are typically binucleate and densely filled with contents, which shows such a strong affinity for the stain that the nuclei are often obscured. The nature of this loosely arranged irregular primordial layer is best shown from a surface view, as in Fig. 6. The material from which this drawing was made was obtained by stripping the epidermis from a

freshly collected plant, immersing it in 95% alcohol for a few seconds, and then mounting directly in lactophenol to which a small quantity of cotton blue had been added. The primordial cells stain very deeply and their dark blue color stands out in sharp contrast to the colorless epidermal cells above. There is a considerable variation in the shape and size of the cells shown in this figure, the majority being very irregular and ranging in size from 12-22  $\mu$  in length to 8-17  $\mu$  in width. A favorite position for the massing of the hyphae, and thus for the formation of primordial cells, is in the intercellular space which lies below the junction of two epidermal cells. In many cases a hyphal strand forces its way through this intercellular space following the outline of the epidermal cells. Each cell of this runner may now enlarge and a row of primordial cells may be formed below the common wall of the overlying epidermal cells (Fig. 7). The primordial cells are not restricted to these intercellular spaces, and may "cover" the underportion of the epidermal cell wall more or less completely (Fig. 6). In this figure 13 primordial cells may be found below the single epidermal cell, the outlines of which are represented by the dotted lines. The number of primordial cells per epidermal cell varies, in some cases there being very few. Where they occur singly they are somewhat oval or club-shaped and possess a fairly regular outline (Fig. 8). The two isolated primordial cells are typical of this stage and show the two characteristic nuclei as well as the dense cytoplasmic contents.

The primordial cell stage is marked externally by a change in the appearance of the hypertrophied stems, which now lose their pale green color and become rose or flesh colored. Earlier workers, as Hartig and Tubeuf, had called attention to this peculiar flesh-colored character, but it had never been correlated in any way with the development of the parasite. As the hyphae mass up under the epidermis and the primordial cells begin to develop, the contents of the epidermal cells begins to disintegrate, and the pale green color of the stem disappears.

The contents of the primordial cells now begins to pass into the cells of the epidermis. The epidermal cell wall which overlies the primordial cell is pierced, and a small opening is formed immediately above the primordial cell through which the contents passes into the interior. These openings are very minute and it is difficult to observe them in this stage, although they may be found very readily in horizontal sections through mature spores. In cross section they appear as small white spots in the epidermal cell wall. As the cytoplasm passes through, a small globular body is formed directly on the cell wall. The youngest stages that have been found are small heavily staining bodies in the epidermal cell (Fig. 9, *a, b*; Fig. 10, *a*). These are called teliospore initials, since each one grows directly into a teliospore. As the initial increases in size (Fig. 9, *c, d, e*) the two nuclei move to the opening and pass through along with the remaining cytoplasm (Fig. 10, *b*).

Each primordial cell gives rise to a single teliospore initial and thus the number of teliospore initials in a single epidermal cell depends upon the number of primordial cells that are formed below it. Where the number is

small, the initials tend to retain their globular shape (Fig. 9, *b, d*; Fig. 10, *b*), but where many are developing in the same cell, the initials become crowded and are somewhat sac-like or elongated (Fig. 10, *c*). It was this sac-like appearance that led to much confusion in earlier studies of *Calyptospora*. In this stage the initials often resemble haustoria, and as haustoria are normally present in the epidermal cells (Fig. 9, *f*; Fig. 10, *d, e*), they were at first thought to be the initials themselves. That this is clearly not the case is shown when the initials are compared with the haustoria. A typical haustorium possesses a well-marked, peg-like process, at the end of which is located the narrow sac-like haustorium (Fig. 11).

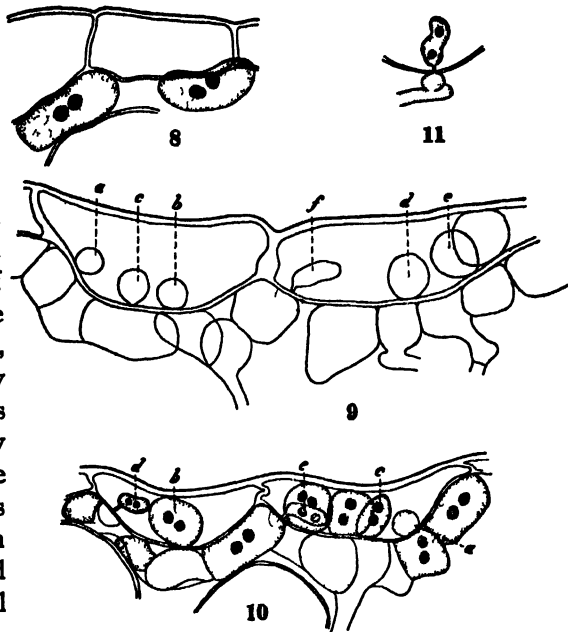


FIG. 8-11. *Calyptospora goeppertiana*. FIG. 8. Two primordial cells.  $\times 620$ . FIG. 9. Teliospore initials at *a, b, c, d, e*. Haustorium at *f*.  $\times 620$ . FIG. 10. Teliospore initials *a, b, c*. Haustoria at *d, e, f*.  $\times 620$ . FIG. 11. Typical haustorium from cortex.  $\times 620$ .

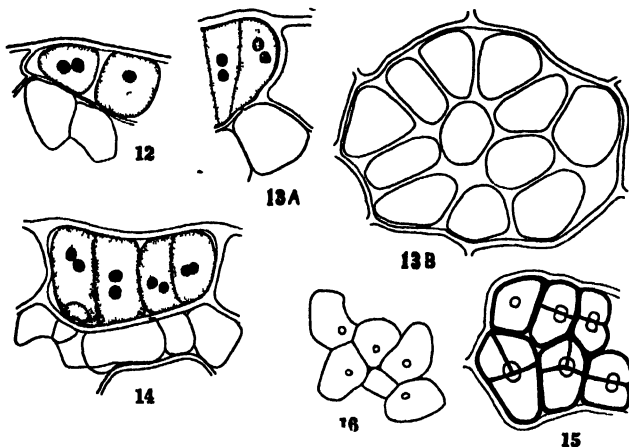


FIG. 12-16. *Calyptospora goeppertiana*. FIG. 12. Two teliospore initials. Note the empty primordial cells below.  $\times 540$ . FIG. 13A. Slightly later stage with opening in the cell wall just above the empty primordial cell.  $\times 630$ . FIG. 13B. Surface view of a single epidermal cell containing 12 teliospore initials.  $\times 540$ . FIG. 14. Cross section of later stage showing an epidermal cell almost completely filled.  $\times 540$ . FIG. 15. Mature teliospores in epidermis showing thickened walls and germ pores.  $\times 540$ . FIG. 16. Group of empty primordial cells showing the opening through which the teliospore initials have arisen.  $\times 540$ .

The teliospore initial, on the other hand, is spherical, seated directly on the cell wall, and appears to arise without any suggestion of a penetration peg.

The teliospore initials increase in size, flattening out against the upper and lower walls of the epidermal cell. The cytoplasm is distinctly vacuolate, the two nuclei are close together and the whole initial is in striking contrast to the large empty primordial cell below (Fig. 12). A surface view of this stage shows that the epidermal

cell becomes more or less filled with these large teliospore initials, which lose their rounded appearance as they conform to the shape of the host cell, and as they become flattened against their neighbors (Fig. 13, B). In this figure 12 teliospore initials may be observed in a single epidermal cell. As further increase in size takes place the nuclei divide conjugately, a cross wall is laid down, and the initial becomes two celled. The opening in the cell wall through which the contents of the primordial cell has passed may occasionally be observed (Fig. 13, A). In Fig. 14 two initials are shown in cross section, and the epidermal cell is more or less completely filled. A number of empty primordial cells may still be observed below this cell of the epidermis, while a small haustorium is discernible at the lower left-hand corner of the cell.

The initials which have divided, or are about to divide, are more properly termed young teliospores, since they may become teliospores without further division. The initial may not even divide but may grow directly into a single-celled spore. In most cases, however, division does take place, and as a result the teliospores are two, three or four-celled. As the spores mature the walls become thickened and dark brown in color. Of the six mature teliospores shown in the surface view in Fig. 15, it will be seen that one is single celled, three are two celled and two are three celled. The germ pores are characteristic, in the two-celled spores occupying a central position on the upper wall, one on each side of the cross wall, and in the three- and four-celled spores they are situated in the upper and inner corner. Each cell contains a fusion nucleus, which is in the resting condition. Below the epidermis the large empty primordial cells can readily be seen, each with a small opening through which the contents has passed into the teliospore initials (Fig. 16).

The number of teliospores contained in a single epidermal cell depends, of course, upon the number of teliospore initials. In Fig. 13 are found 12 initials, which indicates that in this epidermal cell 12 teliospores would be developed. The number of initials depends also upon the number of primordial cells. Below the epidermal cell shown in Fig. 6, 13 primordial cells may be counted. Although the number appears to vary somewhat, it seems that on the average about 12 primordial cells are formed to each epidermal cell, and thus 12 teliospores at the time of maturity.

One of the most striking features of teliospore development is that it is not simultaneous, but begins at the base of the stem and progresses steadily upward. As the teliospores mature the thickened walls give the infected stems a golden brown color, which deepens later so that the stems have a varnished, dark brown appearance. Above this is a narrow light brown zone, which is characterized by the presence of young teliospore initials. The stem, just above this light brown area, possesses the rose color characteristic of the young infected stems, and which we find in the primordial stage referred to previously. Thus, the formation of teliospores in *Calyptospora* is progressive, and there are three zones or areas on the stem; the lower part containing mature spores and dark brown in color; the intermediate zone where

teliospore initials are present, which is light brown in color; the upper part in the primordial stage, and flesh or rose in color. The extent of this intermediate zone is somewhat variable, but is usually rather short. One typical infected twig in this condition was measured; the hypertrophied length was found to be 50 cm., the dark brown area measured 32 cm., the light brown area 8 cm., and the upper portion in the primordial stage 10 cm.

Hartig (11) in his description of the development of the teliospores, indicated quite clearly the massing of the mycelium beneath the epidermal cells where certain hyphae became thickened in a club-shaped manner (see his Fig. 122, *a, a*). These are undoubtedly primordial cells, although they are much smaller than those found by the writer. Hartig has also shown in this figure the presence of haustoria and young teliospore mother cells in the epidermal cells. A slightly later stage (*c*) was shown in one case which corresponds closely with the teliospore initials described above. The teliospore mother cells were thought by Hartig to divide into four teliospores. If the teliospore mother cells of Hartig are considered to be teliospore initials, then the four teliospores are the result of the division of this initial, and are simply the four cells of a single teliospore. From the standpoint of development a teliospore is the product of a teliospore initial regardless of whether the initial divides many times or not at all. In *Calypsozona* the initials usually divide but the teliospores have not been found to exceed more than four cells.

## 2. *Milesia*

Among the genera that are included in the Pucciniastreae are three which comprise the common fern rusts of temperate regions, namely *Milesia*, *Hyalopsora* and *Uredinopsis*. Although Moss (16) has studied the uredo stage of these genera, very little is known of the development of the teliospores. The results of investigations made in this connection on *Hyalopsora* are being described in a separate paper (18), while the studies that have been made upon *Uredinopsis* will be described later in this paper. It is with the genus *Milesia* that the writer is at present concerned. In a recent monograph of this genus, Faull (6) has discussed the taxonomy and geographical distribution, and has also reviewed the investigations of earlier workers. In his discussion of the taxonomy Faull has concluded that *Milesia* White should be the proper name for the genus, and *M. polypodii* White the type species. In this paper the generic name will be used as defined by Faull.

The teliospores of *Milesia* are intradermal, as in *Calypsozona*, but in this case the spores are formed in the epidermal cells of the old overwintered fronds. Earlier workers looked for teliospores during the current season, and considered them to be of rare occurrence. The explanation is that in most cases they were entirely overlooked. As Faull has pointed out, "this is not surprising, because they bear little resemblance to spores, and until one knows when and where to look for them they are not likely to be seen." In his monograph covering 33 species, teliospores are described for 22 species and varieties. Kamei (12), in a note on *Milesia vogesiaca*, has given two



figures of the mature teliospores. One figure was a cross section through two epidermal cells showing the intradermal teliospores and below the epidermis, mycelium with a few small empty rounded hyphal cells. In a later paper (13) Kamei described and figured a new species, *M. jezoensis*, in which the same type of cell was shown beneath the epidermis.

In the Lake Temagami region where the species included in this paper were collected and studied, there appear to be four species of *Milesia*: *M. marginalis*, whose teliospores develop in the spring on the overwintered fronds of *Thelypteris marginalis*; *M. polypodophila* and *M. intermedia*, which are similar in time of development, and which are found on *Polypodium virginianum* and *Thelypteris spinulosa intermedia* respectively; the fourth species, *M. fructuosa*, is found in the fall on the season's leaves of *Thelypteris spinulosa americana*. Although the bases on which *M. fructuosa* and *M. intermedia* have been separated might be open to question, they will be referred to in this paper as acceptable species. The development of the teliospores has been studied in all four species and the results indicate that there is a striking similarity in the manner of development in all of them. It was accordingly thought best to illustrate the manner of development by reference to only one species, and *M. marginalis* was therefore selected for detailed study. Such differences as may have been found in the other species will be indicated in the course of the paper.

The appearance of the infected parts of the overwintered fronds in which the teliospores will be formed is very characteristic. In *M. marginalis* these infected patches are usually light brown or tan in color, and extend over a considerable area. There is also much variation in the size of these light brown patches, from small limited portions to areas involving half the pinnule. A peculiar feature is that infection is often found on only one side of the main vein, although basal infections are sometimes extended over both sides. The usual appearance, however, is a well-defined light brown area of varying size on either side of the main vein. There is also considerable physiological browning, which tends to obscure the areas of infection. This type of browning is usually not localized but involves a number of pinnules.

In *Milesia polypodophila*, the infected leaves show dark brown localized patches at the margins or tips of the pinnae. The epidermis usually becomes separated from the cells below, and may be readily stripped off. Whether this is due entirely to the rust mycelium or to senescence of the leaf, or both, has not been determined. As in *M. marginalis*, there is often considerable browning that is not due to the rust infection, and which tends to obscure the part of the leaf in which teliospores are developing. In this connection the presence of uredinial pustules was a valuable aid in discovering these areas, due to the fact that they are usually found in association with the teliospores. In *M. intermedia*, on the other hand, the uredinia rarely have been found. The lesions in which the teliospores are produced are rather extensive in this species, and are brown in color. *Milesia fructuosa* causes large dark blotches on the fronds, which may extend over several pinnules.

Sections through these infected areas indicate the presence of abundant mycelium throughout the intercellular spaces of the mesophyll. The mycelium is composed of rather large hyphal cells, each with two nuclei. The first indication of the beginning of teliospore formation is in the aggregation of hyphae below the epidermal cells. This phase is not as prominent as in *Calyptospora*, where a great many hyphae are massed below the epidermis. In *M. marginalis* fewer hyphae take part in the process, while occasionally only a single mycelial strand is found.

The formation of primordial cells in all *Milesia* species is similar in every way to the process described earlier for *Calyptospora*. The simplest type is where a hyphal tip becomes greatly enlarged and is cut off to form the primordial cell. More often, however, the origin of the primordial cells is from a hyphal runner, which is applied closely to the epidermis (Fig. 17). In the hyphal runner shown in this figure the cell at the tip has become cut off to form a primordial cell, while the cells of the strand are much enlarged, and will undoubtedly break up into other primordial cells. Two branches are given off this runner immediately below the side walls of the overlying epidermal cell. When the epidermis is stripped off, the primordial cells are often found in this position, indicating that they have arisen from such hyphal runners.

The mature primordial cells are typically binucleate and filled with cytoplasm, which is usually vacuolate (Fig. 18). The nuclei are very prominent, owing chiefly to the presence of a single large nucleolus. The primordial cells are larger than in *Calyptospora*, and often have a length of 28  $\mu$  and a width of 15  $\mu$ , occasionally being even larger.

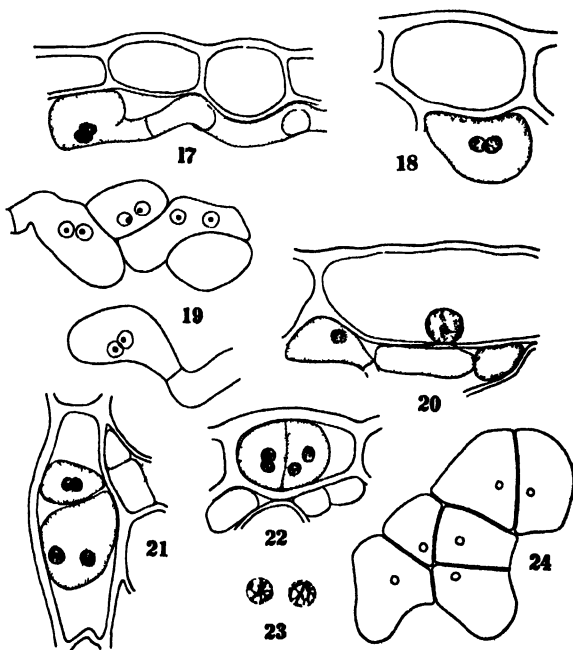


FIG. 17-24. *Milesia marginalis*. All figures  $\times 600$ . FIG. 17. Hyphal runner below lower epidermis. Individual cells much enlarged and the tip rounded up to form a primordial cell. FIG. 18. Cross section through an epidermal cell with a mature primordial cell below. FIG. 19. Surface view of a group of primordial cells. The four upper cells are somewhat irregular in shape, while the single one just below is club-shaped. FIG. 20. A young teliospore initial arising from the central primordial cell. FIG. 21. Two initials, the lower one being much larger. Note the empty primordial cells in the small intercellular space just below the epidermal cell. FIG. 22. Two-celled stage. FIG. 23. Two fusion nuclei from two cells of a mature teliospore. FIG. 24. A mature teliospore with six cells, whose shape corresponds to that of the epidermal cell. A germ pore is present on the upper wall of each cell.

Single primordial cells are somewhat club-shaped and are applied closely to the epidermis (Fig. 18). Where the primordial cells occur in groups they are somewhat smaller in size and are usually irregular in shape. This is well illustrated in Fig. 19, which is a surface view of a group of five primordial cells. The upper four tend to be somewhat irregular, while the lower single one is regular and club-shaped.

In *Milesia marginalis* the primordial cells are formed more abundantly beneath the cells of the lower epidermis, than below the upper epidermis. The cells of the mesophyll are more compact in the upper part of the leaf, and the intercellular spaces are less abundant and often much smaller. The primordial cells, as a result, are very irregular in shape and much smaller in size. This characteristic of conforming to the shape and size of the intercellular space has been found to be true of *Hyalopsora* (18), and is probably found in all species which possess intradermal teliospores.

The number of primordial cells produced is not large. When the epidermis is stripped off small groups can be found, with here and there single cells. Since the number of teliospores is proportional to the number of primordial cells, the number of epidermal cells containing teliospores should give some clue to the number of primordial cells produced. In a surface of 15 epidermal cells 13 were found to contain teliospores, and two were empty. Of these 13, one cell contained four teliospores and was completely filled, while in the remaining 12 there were from one to three spores which filled only part of the epidermal cell. Thus, in the 15 epidermal cells there were approximately 22 teliospores, which indicates the presence of 22 primordial cells. The number of teliospores per epidermal cell however varies greatly, and a single epidermal cell may have as many as five or six, or may have but a single one.

In *Milesia intermedia* the primordial cells are much smaller, and there is a tendency for them to become somewhat irregular. *Milesia polypodophila*, on the other hand, possesses primordial cells which agree very closely with the description given for *M. marginalis*. The primordial cells of *M. fructuosa* are somewhat different from the other species. They are much elongated and usually very narrow. Where they are crowded by the host cells they conform to the shape of the intercellular space, and may be much smaller. In all cases, however, two nuclei are present and the contents is filled with dense cytoplasm.

The passage of the contents from the primordial cell into the host and the formation of teliospore initials have been studied in all four species. The first indication of this process is seen in the formation of small round globular structures in the epidermal cell immediately above the primordial cells. As the contents passes in, these globular bodies increase rapidly in size and it is very difficult to find the first stages. Fig. 20 is an early stage of *M. marginalis* showing three primordial cells and a teliospore initial in the epidermis which has arisen from the central one. Much of the cytoplasm has already passed in and the initial has stained so deeply that it is impossible to make out the

nuclei. These stages have been observed in the other three species, and they agree very closely with *M. marginalis*. *Milesia fructuosa* is especially favorable for the study of the early stages of the teliospore initials.

The young teliospore initial grows rapidly as the contents flows in, and assumes a spherical or slightly oval shape. Where two or three are growing side by side there is usually considerable crowding and the shape of the initial is often altered. In Fig. 21 two initials are shown, the upper one of which is filled with contents and stains very heavily, while the lower is much older and the cytoplasm is vacuolate. In both there is a slight tapering toward the base. This tapering in *M. intermedia* is found even where there is but a single initial in the cell. The nuclei in the teliospore initial now divide, and we have a two-celled stage, each cell with two prominent nuclei (Fig. 22). Further division takes place resulting in the formation of many-celled teliospores, completely filling the epidermal cell. Since these spores conform to the shape of the epidermal cell, and also to the presence of neighboring spores, it becomes very difficult to distinguish just how many cells belong to a particular spore. According to Sydow (20) it is not easy to determine the exact size and extent of the single spores. In some cells of the epidermis there appear to be four cells in isolated groups, each group being the result of two divisions of the teliospore initial. In other cases the number of cells is much greater. This may be due to the presence of more than one primordial cell or to a greater number of divisions of the teliospore initial. The final result is that the epidermal cell becomes filled with multicellular thin-walled teliospores, which conform to the shape of the epidermal cell, completely filling it. Below the epidermal cells the empty primordial cells may be found. Kamei's figures are of this stage and his empty hyphal cells are undoubtedly empty primordial cells.

Each cell of the teliospore contains two nuclei, each with a prominent nucleolus. As the teliospores mature, the two nuclei approach each other and a large fusion nucleus results. The details of the nuclear fusion have not been studied, but the following stages may be noted. In *M. fructuosa* the fusion nucleus immediately goes into a resting condition, the chromatin being in the form of very fine granules, and the whole nucleus has a homogeneous appearance. The two nucleoli do not fuse immediately, and both are present in the resting stage, which is of brief duration. A spireme thread is evolved, and sections through mature teliospores indicate quite clearly that the fusion nucleus is not in a resting condition, but is always found in prophase. Two typical fusion nuclei are shown in Fig. 23. In the nucleus at the left a single large nucleolus is present, which is probably the result of fusion. In all four species of *Milesia* the fusion nucleus of the mature teliospore has been found to be in this spireme stage.

In a separate paper (18) attention has been drawn to the nuclear condition in the teliospores of *Hyalospora aspidiotus*. The nuclei came together and immediately passed into a short resting period. The fusion nucleus then entered into heterotypic prophase, and a well-defined spireme, was evolved

which was shown to be double. The nuclei of the mature spores were therefore in an advanced stage of prophase, and not in a resting condition. This stage was of long duration, as the remaining stages took place in the promycelium when the spores germinated. Although all the stages have not been observed in *Milesia*, the number that have been found are sufficient to indicate that we have a situation in *Milesia* which is comparable in every way with *H. aspidiotus*.

Each cell of a teliospore possesses a well-marked germ pore. In Fig. 24 a single teliospore is shown in surface view, which is composed of irregular cells, each with a germ pore in the upper wall. This group of cells conforms to the general shape of the containing epidermal cell, which is not indicated in the drawing. A comparison of this figure with Fig. 15 of *Calyptospora* indicates quite clearly the difference in spore structure and in germ pores. In *C. goeppertiana* the teliospores are thick walled and germinate the following spring; in *Milesia*, as in *Hyalopsora*, they are thin walled and germinate at once. The germ pores of *Calyptospora*, which are located at the upper and inner corner of each cell, indicate their origin from a teliospore initial. In *Milesia*, however, the germ pores are located on the upper wall, but at no particular point in so far as the teliospore initial is concerned.

### 3. *Thecopsora*

*Thecopsora vacciniorum* occurs on the leaves of *Vaccinium pennsylvanicum* and *V. canadense*. It is found fairly abundantly on the under side of the leaves as small whitish spots, which become brown as the teliospores mature. At Lake Temagami in northern Ontario, where this rust was studied, the teliospores begin to develop toward the end of the growing season, and all stages may be found during September. The teliospores are intradermal and are found in both the upper and lower epidermis. This character led Magnus to separate from *Pucciniastrum* all those species whose teliospores are not subepidermal, but are found in the epidermis, and on this basis he created a new genus, *Thecopsora*. Dietel (4) in his recent treatment of the Uredinales, has retained this generic name, and has brought together all the genera whose teliospores are borne in the epidermal cells. This grouping includes *Calyptospora*, *Milesia*, *Hyalopsora* and *Thecopsora*, all of which have been studied from the standpoint of teliospore development. It has been found that *Thecopsora* is similar to the above three genera in the manner in which the teliospores are formed. From the standpoint of development, it would seem that *Thecopsora* should be separated from *Pucciniastrum*, and the generic name has accordingly been used throughout this paper.

During the growing season the rust is present in the uredinal stage and the mycelium becomes well established in the leaf. In September the primordial cells are formed and from them the teliospores are developed. The palisade layer is very compact and intercellular spaces are not abundant, while the loosely arranged mesophyll beneath the lower epidermis, on the other hand, is provided with a great many air spaces. It is in these spaces between palisade cells or between cells of the mesophyll that primordial cells are developed.

The first indication that can be found of primordial cell development is the slight enlargement of hyphal cells in the intercellular spaces just below the epidermis. These enlargements are not as prominent as in *Milesia* or *Calypotspora*, since the mycelium in the leaf is composed of rather large hyphal cells, which do not differ greatly from young primordial cells.

The mycelium is most abundant in the mesophyll, where the intercellular spaces are large. In order to reach the upper epidermis, the hyphae force their way between the palisade cells, and when the epidermis is reached they flatten out against the wall of the epidermal cell, the whole tip expanding slightly, conforming to the intercellular space. The cytoplasm becomes very dense and two nuclei are regularly present (Fig. 25). This type of primordial cell is found only when the tissues of the host are very compact. Below the lower epidermis certain hyphal cells, in many instances the end

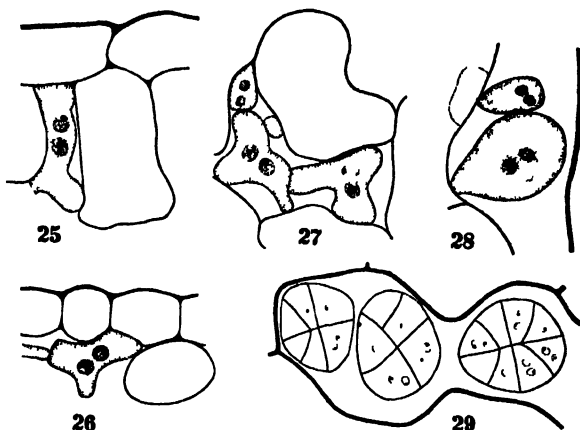


FIG. 25-29. *Thecopsora vacciniorum*. All figures  $\times 610$ . FIG. 25. A primordial cell below the upper epidermis. FIG. 26. Primordial cell beneath lower epidermis. Note the difference in shape when the primordial cell lies in a large intercellular space. FIG. 27. Two characteristic primordial cells in an intercellular space in the mesophyll immediately beneath the lower epidermis, shown in cross section. FIG. 28. Two teliospore initials in a cell of the upper epidermis. The smaller initial is densely filled with cytoplasm. The initial just below is a later stage. FIG. 29. Surface view of a part of a cell of the upper epidermis, containing three multicellular teliospores.

cell of a mycelial strand, enlarge to form small irregular binucleate primordial cells (Fig. 26). A comparison of Figs. 25 and 26 illustrates quite clearly the two different types of primordial cells, and also gives some indication of the difference between the structure of the upper and lower epidermis.

The number of primordial cells that are formed is not large. Horizontal sections through the infected areas indicate that the number of primordial cells per epidermal cell is usually small. This is especially true of the upper epidermis where the number is small in comparison with the lower epidermis, where they are much more abundant. Two typical primordial cells are shown in Fig. 27, which is a horizontal section through the mesophyll beneath the lower epidermis. Mature primordial cells are filled with contents which show a strong affinity for stain, and these are always binucleate, each nucleus having a prominent nucleolus.

The contents of the primordial cell now passes through the lower wall of the epidermal cell, the passage of the contents taking place very rapidly, as in *Milesia* and *Hyalopsora*. The youngest stage that could be found is shown in Fig. 28, which illustrates two teliospore initials. The upper one is small

sac-like and binucleate, with dense contents which has just passed in from the primordial cell. Increase in size takes place and the teliospore initial becomes greatly enlarged. In the upper epidermis the initial becomes somewhat obovate in shape (Fig. 28), while in the lower epidermis the cells are much smaller and the initial soon conforms to the shape of the host cell. The cytoplasm is very vacuolate and the two nuclei are prominent.

The nuclei in the teliospore initial begin to divide and vertical septations are laid down, giving a multicellular teliospore. The first two septations are usually at right angles to each other and divide the initial into four cells. Where the cross walls meet at the centre of the spore, there is usually a well-defined polar furrow. These septations, however, do not necessarily divide the initial in a symmetrical manner, and the spore may have a very irregular appearance. This is especially marked in the teliospores that are formed in the lower epidermis where the cells are much smaller and narrower than those of the upper epidermis, and the initials during growth conform to the adjacent cell walls so that the mature teliospores are very irregular, often filling the cells. Where there are fewer initials in a cell, and where spacial relations permit, the teliospore tends to retain a definite outline.

In the larger cells of the upper epidermis there is sufficient room for the teliospore initials to develop, and the teliospores have a rounded outline. Since the initials are few in number, the teliospores of the upper epidermis are scattered, or in small irregular groups. In 19 epidermal cells examined six possessed a single teliospore; in nine others there were two, in two there were three, while the two remaining cells each contained four teliospores. As the spores mature, septations are laid down in a very irregular fashion. Three typical teliospores from the upper epidermis are shown in Fig. 29. The spore at the left contains six cells, the central one five, while the spore at the right is eight celled, and is bilaterally symmetrical. In any group of spores from the lower epidermis there is a greater variation in shape, size and number of septations. Two nuclei are present in each cell of the teliospore. These nuclei probably fuse later, although fusion has not been observed.

#### 4. *Pucciniastrum*

In the Temagami region of northern Ontario a number of species of *Pucciniastrum* have been found. Of special interest are five species, namely, *P. abieti-chamaenerii*, *P. epilobii*, *P. pyrolae*, *P. potentillae* and *P. agrimonae*. Two other species are present in abundance, *P. americanum* and *P. arcticum*. Teliospores were found in only four of the above seven species. These four species have been studied and it has been found that *P. epilobii*, *P. americanum* and *P. arcticum* are very similar in the method of teliospore development, while *P. abieti-chamaenerii* differs from all three. As a result, *P. abieti-chamaenerii* and *P. epilobii* have been selected as representative and will be described in detail.

*Pucciniastrum abieti-chamaenerii* is found on *Epilobium angustifolium*, while *P. epilobii* occurs on *E. adenocaulon*. The teliospores of both species are formed late in the growing season, and collections were made during

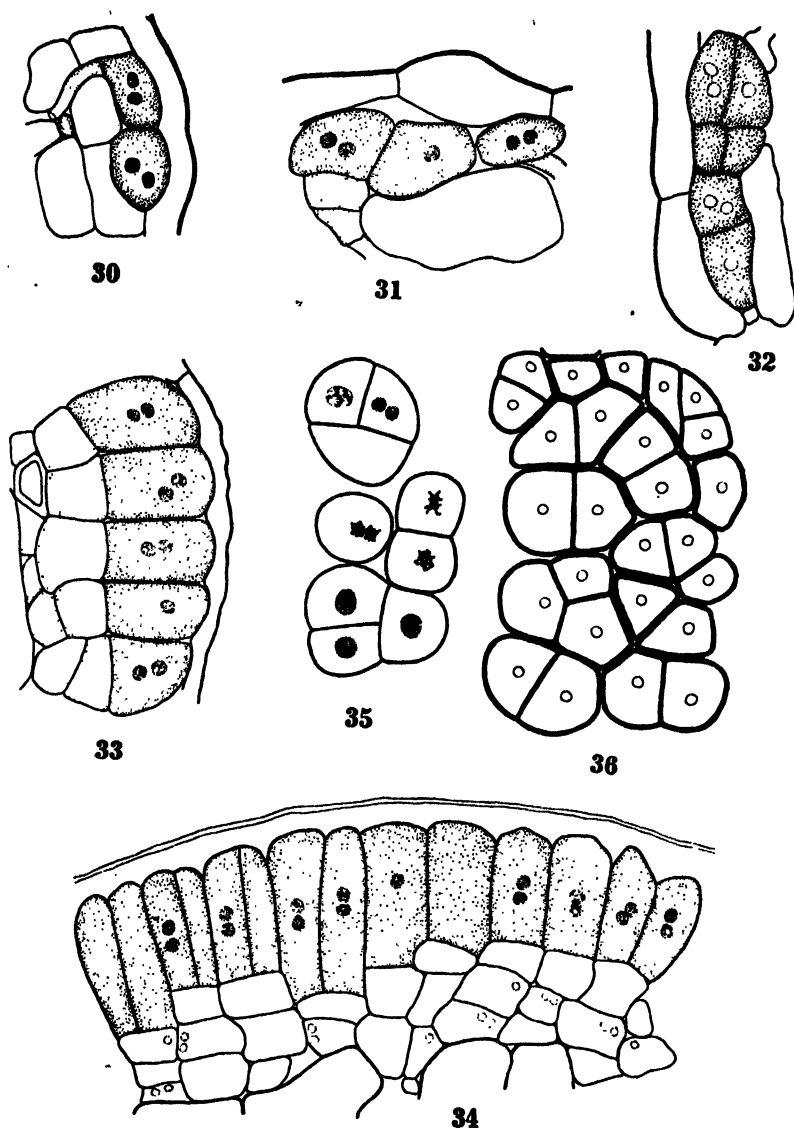


FIG. 30-36. *Pucciniastrum*. All figures  $\times 720$ . FIG. 30. *P. epilobii*. Two young primordial cells below the epidermis, which is indicated by a heavier line. FIGS. 31-34. *P. abieti-chamaenerii*. FIG. 31. A median section through a group of primordial cells. FIG. 32. Four primordial cells, the upper two of which have undergone division. FIG. 33. Later stage showing growth in a vertical direction, and also empty basal cells. FIG. 34. A section through a group of teliospore initials, which are the terminal cells of a vertical row. Five of the initials have divided, laying down vertical septations. All cells, except the teliospore initials, are practically empty. FIG. 35. *P. epilobii*. A horizontal section through a number of teliospores, showing different nuclear conditions at the time of fusion. The mature teliospores have a single fusion nucleus. FIG. 36. *P. abieti-chamaenerii*. Horizontal view of the edge of a large group of mature teliospores with thick cell walls and germ pores.



September and October. In *P. abieti-chamaenerii* they form extensive crusts which appear on the under side of the leaves as blackish-brown areas. The teliospore layer in *P. epilobii* is much smaller. The mature teliospores, which are well described by Sydow (20), are thick walled and are always subepidermal, and in this respect differ from the teliospores of *Calypsotheca*, *Milesia* and *Thecopsis*, which are intra-epidermal. From the standpoint of development also, *Pucciniastrum* has been found to be considerably different.

During the growing season the mycelium is well developed, and often fills the intercellular spaces of the leaf. Toward the end of August sections through infected parts of the leaf indicate that certain hyphae force their way between the cells of the mesophyll just beneath the lower epidermis. The tissues in the *Epilobium* leaf are very compact and the hyphae, in growing toward the epidermis, separate the mesophyll cells as they grow between them. When they reach the lower epidermis, they tend to flatten out against the lower wall. The end cell of the hypha becomes cut off and begins to increase in size. Fig. 30 shows two very young primordial cells of *P. epilobii*, the upper one being the terminal cell of the hyphal strand. As growth takes place the epidermis becomes slightly raised. The contents of the primordial cells stains very deeply and it is difficult to make out the details of the two nuclei which are present.

In *Pucciniastrum abieti-chamaenerii* the primordial cells are formed in a somewhat similar fashion, except that a great many hyphae are involved, and the formation of primordial cells is not necessarily a property of the terminal hyphal cells. While in a few cases primordial cells are solitary or in a small group, in most cases a great many are formed from a hyphal complex just beneath the lower epidermis. Fig. 31 is a section through the middle of a group of primordial cells in various stages of development. On either side of this median section are other primordial cells, which are smaller toward the outer edge of the group. It is clear that these have arisen from a web of hyphae, a few strands of which may still be seen. At the centre of the group the cells are large and closely packed, while toward the edge they tend to be smaller and more diffuse. The mature primordial cells of *P. abieti-chamaenerii* may reach a considerable size (Fig. 31) as compared with those of *P. epilobii* (Fig. 30). In both species, however, the primordial cells consist of enlarged thin-walled binucleate hyphal cells which are densely filled with coarse granular cytoplasm.

In *Pucciniastrum abieti-chamaenerii* the nuclei undergo division and a septum is laid down in a plane that is parallel to the leaf surface, so that the primordial cell is divided into two cells, which are at right angles to the epidermis. In Fig. 32 four cells are shown, the upper two of which have just divided. This material was poorly stained and it was difficult to make out the nuclei in all the cells. After division the cell adjacent to the epidermis expands greatly, while the lower cell apparently degenerates, since in the next stage these cells are practically empty. Division of the primordial cells takes place more or less simultaneously, and a compact group of cells is developed,

the upper ones expanding vertically so that their length becomes almost double their width. They are binucleate, filled with contents, and furnish a striking contrast to the empty cells below (Fig. 33). As growth continues the upper cells may divide again in the same plane, cutting off below a second cell. Such a case is shown in the lower two cells of Fig. 33, where two empty cells are shown below each of the upper ones. There is often a third division, and another septum is laid down in the same plane, resulting in three basal cells in a row. In every case the contents degenerates so that the cells appear to be empty. Thus we have a series of cells which have arisen by repeated division of the primordial cells, and these divisions, being in the same plane, have resulted in a row of cells. Since the contents of all cells except the terminal one degenerate, this upper cell is thus the potential teliospore. Earlier in this paper the term "teliospore initial" has been used to indicate the cell which gives rise directly to the teliospore. Since this terminal cell becomes the teliospore it may be referred to as the teliospore initial.

The initial may give rise to a teliospore without further division, or it may divide once to give a two-celled spore, or twice to give a four-celled spore. The division of the teliospore initial takes place in a different plane from that of the primordial cell. The axes of the spindles instead of being at right angles to the leaf surface are parallel to it, and the septum that is laid down is a vertical one. In Fig. 34 nine teliospore initials are shown, each with a row of two or three empty cells below. In five of these initials vertical septations have been laid down, and the walls of each cell have become slightly thickened.

*Pucciniastrum epilobii* differs from *P. abieti-chamaenerii* in the absence of a row of empty cells below the teliospore. The primordial cell usually divides only once, the upper cell becoming the teliospore initial. The lower cell is prominent in the early stages but later the contents begins to disappear, although the two nuclei may usually be found. The teliospore initial divides, laying down vertical septations, as in *P. abieti-chamaenerii*, and the mature spores are one- to four-celled. Two nuclei are present in each cell of the teliospore. As the spores mature the nuclei come together and fuse. Various stages in this process of fusion are shown in Fig. 35. At first the two nuclei are small and homogeneous, but as they move together the chromatin is heavily staining and each nucleus has an irregular outline. The fusion nucleus is often to be observed in a spireme stage, and two cells have been drawn illustrating this. In many of the spores there is a large nucleus which appears to be in a resting condition. Whether this precedes spireme formation, as in *Hyalopsora*, is not known, but it is probable that since the teliospores do not germinate immediately, the nuclei overwinter in a resting condition.

The teliospores of *Pucciniastrum abieti-chamaenerii* develop a thickened light brown wall, which may be 3  $\mu$  in thickness, according to Grove (10). In the upper part of each cell there is a thinner spot, the germ pore, which shows very clearly from a surface view. In Fig. 36 a group of mature teliospores has been drawn, each cell showing a germ pore in the upper wall. This

group comprises a few cells of the margin of a large extended crust of spores. The outermost cells are at the left-hand side of the drawing, and tend to be more rounded or regular than those in the centre of the group, which are very compact. This difference has also been noted by Grove (10) in his description of the mature spores . . . "those in the middle of the sorus *Melampsora*-like, but at the periphery roundish or oval, and composed of 1-3 cells, i.e., divided by longitudinal walls." The teliospores do not always occur in such extended crusts, but may be found in small isolated groups, or even singly. In the latter case the spores are regularly four celled, not three celled, as Grove has described.

### 5. *Uredinopsis*

*Uredinopsis* is the third genus of fern rusts that belong to the Pucciniastreae, and is probably the best known. Faull (5), in his study of the morphology, biology and phylogeny of these genera, reviewed the situation in *Uredinopsis* and considered that this is the most primitive genus. Bell (3) has pointed out a misconception in the distribution of the teliospores. Earlier workers had described them as being distributed throughout the mesophyll, but Bell found that they were situated just below the epidermis, particularly the lower epidermis, where they formed a layer. Little is known, however, of the manner in which the teliospores are formed, and if this rust is to be considered as primitive then such a developmental study might be expected to throw some light upon this question.

Five species have been found in the Temagami Forest Reserve, namely *U. osmundae* on *Osmunda claytoniana*, *O. regalis* and *O. cinnamomea*; *U. struthiopteridis* on *Pteretis nodulosa*; *U. atkinsonii* on *Asplenium filix-femina*; *U. phegopteridis* on *Thelypteris dryopteris*, and *U. mirabilis* on *Onoclea sensibilis*. The teliospores of all five species were collected in abundance during August and September, and material showing various stages in development was obtained. In the species that have been studied it has been found that there is a great similarity in the method of teliospore development. *Uredinopsis phegopteridis* has been selected and a detailed description will be given of this species. A few figures of *U. struthiopteridis* have also been included for the sake of comparison.

In *Uredinopsis phegopteridis* the infection is bounded by the vascular bundles of the leaf, and thus the area of a lesion is that of a leaf islet. Sections through these areas reveal occasional uredinia with numerous white spores. The character of the uredospores readily distinguishes this species from infections of *Hyalopora*, which are very similar, but which have orange-colored uredospores. The structure of the leaf of *Thelypteris* is very simple, there being no palisade layer, while the mesophyll cells are large in size with abundant intercellular spaces. In the infected areas the mycelium is most abundant in the intercellular spaces just below the epidermis, and the first indication of teliospore formation is found in this region. The tip of a hyphal strand comes to lie immediately below the epidermal cell, and applied closely to it. A septum is laid down, cutting off the tip, which contains two nuclei

and considerable cytoplasm. These terminal cells are enlarged, in the majority of cases tending to be somewhat club-shaped, and these are the primordial cells. In Fig. 37 a single primordial cell is shown, immediately beneath the upper epidermis. The primordial cells are usually fewer below the upper epidermis, being found most abundantly beneath the lower epidermis, although they are occasionally to be found in the mesophyll. In *U. struthiopteridis* the primordial cells are similar, being formed by the enlargement of the terminal cell of a hyphal strand. Fig. 38 illustrates a primordial cell of *U. struthiopteridis* beneath one of the cells of the lower epidermis. This is a later stage than in Fig. 37, and indicates the beginning of the rounding up of the primordial cell, which is the next stage in development. These rounded cells are found in abundance in a heavy infection, and are readily recognized by their dense contents and definite outline.

The rounded cells are potentially teliospores and since they have each arisen directly from a primordial cell they may be properly termed teliospore

initials. The initial undergoes rapid growth, increasing greatly in size, and remaining close to the overlying wall. The appearance of this stage is well illustrated in the two initials that have been drawn in Fig. 39. The upper initial, which lies below the upper epidermis, is small and rounded, while beneath the lower epidermis is a somewhat larger initial in a later stage. The loosely arranged character of the mesophyll cells of the host is also shown in this figure. When the initials reach a certain size the nuclei undergo division and the two pairs of daughter nuclei move to the opposite ends (Fig. 40). After division has been completed a cross wall runs in and

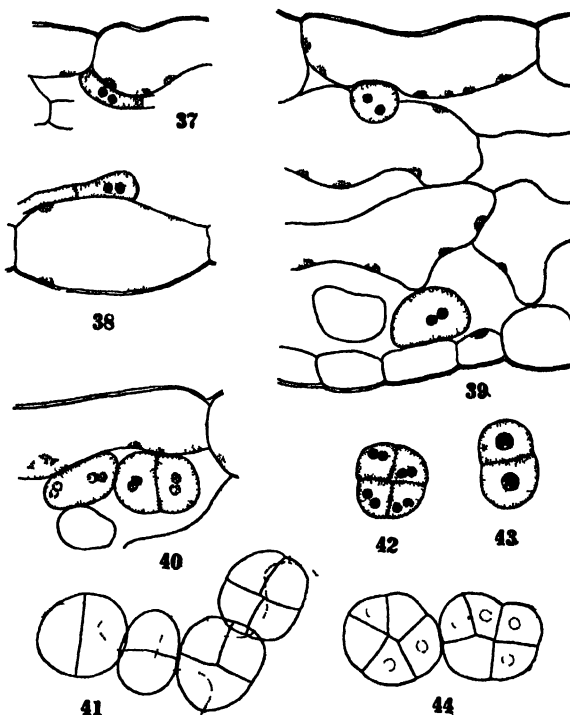


FIG. 37-44. *Uredinopsis*. All figures  $\times 600$ . FIG. 37. Primordial cell of *U. phegopteridis* beneath the upper epidermis. FIG. 38. Primordial cell of *U. struthiopteridis* beneath the lower epidermis. FIGS. 39-43. *U. phegopteridis*. FIG. 39. A section through a frond showing two teliospore initials. FIG. 40. Division of the teliospore initial. FIG. 41. A group of young teliospores in the intercellular spaces below the epidermal cell walls, represented by dotted lines. FIG. 42. A typical four-celled teliospore. FIG. 43. A two-celled spore, each cell with a fusion nucleus. FIG. 44. Two multicellular teliospores of *U. struthiopteridis*.

the initial becomes two celled (Fig. 40). This cell is more properly termed a young teliospore, since it may mature without further division, thus giving rise to a two-celled teliospore. More often, however, the nuclei divide a second time and the teliospore may become either three celled or four celled. The mature spores of *U. phegopteridis* have never been found to contain more than four cells. Four typical teliospores are drawn in Fig. 41, and it will be noted that of these four, two are two celled, one is three celled, and the remaining one is four celled.

The intercellular spaces lying below the lateral walls of the epidermal cells are favorite places for teliospore formation in *U. phegopteridis*. The primordial cell in Fig. 37 is developing in just such an intercellular space. The four spores that are drawn in Fig. 41 are also lying below the side walls of the epidermal cells, the walls being represented as dotted lines. This group is part of a larger group, and if the drawing were extended in either direction it would be found that the majority of the spores would lie beneath the lateral walls. In a prepared section, two nuclei are found in each cell of the mature teliospore (Fig. 42). Although fusion has not been observed a large fusion nucleus is later formed. There is some indication that the nuclei of the teliospore in Fig. 43 have just fused, but in most cases the nuclear details are difficult to observe. In material that has been collected late in the growing season each cell contains a single nucleus, which appears to be in a resting condition. The mature teliospores of *U. struthiopteridis* are multicellular, and very irregular, each cell containing a single fusion nucleus (Fig. 44). The teliospores of *U. osmundae* are similar in every way to those of *U. struthiopteridis*. They are large, many celled and display a wide variation in shape, size and number of cells. This species furnishes an excellent example of the so-called polar furrowing in the arrangement of the septations of the mature teliospores.

### Discussion

From a phylogenetic standpoint the Pucciniastreae have been regarded as primitive rusts and the fern rusts as the most primitive genera. Since the rusts show a high degree of specialization and have presumably evolved along with their hosts, we would expect to find the more primitive rusts upon those hosts which have evolved the least, namely the Pteridophytes. Arthur (1) considered the fern rusts to be primitive and based his argument upon the assumption that "the rusts on the most ancient line of hosts would be likely to reveal more primitive features than those on hosts of later origin." Upon the oldest family of rust-bearing ferns, the Osmundaceae, there was a species of *Uredinopsis*, parasitic on *Osmunda*, which Arthur considered to be the most primitive genus. The genera *Hyalopsora* and *Milesia* were found on various hosts of the Polypodiaceae and were considered to show an advance over *Uredinopsis*. Faull (5) has discussed the Pucciniastreae from a phylogenetic standpoint and has placed *Uredinopsis* as the most primitive rust, with *Milesia* closely related. The evidence upon which these assumptions are made is based primarily upon a study of the morphological features of the spore forms of the various genera concerned.

Despite the fact that in the Pucciniastreae there is a wide variation in the type of teliospore formed, it is significant that in the stages of development of both the intra-epidermal and subepidermal forms there is a general situation that appears to be common to all. The mycelium cuts off certain hyphal cells, which by growth and enlargement become the primordial cells. The primordial cell gives rise to a teliospore initial, which is intercellular in the species possessing subepidermal teliospores, and intracellular in those species which have the teliospore inside the epidermal cells. In the former the initials arise either directly by the rounding up of the primordial cell or after a number of divisions, while in the latter the initial is formed by the penetration of the wall of the epidermal cell, and the passage of the contents into the interior. By growth and division the initial gives rise to a multicellular teliospore, which may be thin walled or thick walled and which may germinate with or without a period of rest. The mature teliospores may be solitary or in groups, forming small to extended crusts; if in the epidermal cell, they may be scattered or in groups completely filling the cell. In all the species that have been studied, the teliospore is the product of a single primordial cell, whether it be many celled or few celled.

In the formation of primordial cells there is some variation in the species that have been studied. In *Calyptospora goeppertiana* the epidermis is separated from the cortex by the large number of primordial cells produced. A surface view indicates that the epidermal cells may be fairly completely "covered" by the large irregular closely packed primordial cells. In *Milesia*, however, the number of cells is greatly reduced and they occur in small groups or more often singly. They are also much larger than those of *Calyptospora*. There are two kinds of primordial cells in *Thecopsora vacciniorum*. The first are large and very irregular, and are found beneath the lower epidermis where the intercellular spaces are large. The other type of cell is narrow and elongated and is formed below the upper epidermis where the primordial hyphae force their way toward the epidermis through the compact palisade layer. They are accordingly at right angles to the leaf surface with the upper end flattened against the lower wall. The primordial cells of the two species of *Pucciniastrum* on *Epilobium* are subepidermal, small and somewhat flattened in cross section. In *P. epilobii* they form loosely arranged groups of varying size, while in *P. abieti-chamaenerii* they are more abundant forming large extended crusts, which appear to arise from a hyphal complex. The genus *Uredinopsis* possesses a comparatively simple type of primordial cell development. The tip of a hyphal strand growing below the epidermis becomes cut off and this cell enlarges somewhat to become the primordial cell. These club-shaped cells are characteristic of all the species of *Uredinopsis* that have been studied.

The teliospore initial in the intra-epidermal species arises directly from the primordial cell by the penetration of the host wall, and the passage of the contents through the wall to form a rounded or sac-like body. The initial appears to become independent since it now possesses the entire cell

contents and in its further development loses all connection with the primordial cell. In *Calyptospora* the initials are small and rounded with many in a single cell. In *Milesia* they are similar to those of *Calyptospora*, except that they are formed in the epidermis of the leaves, and are fewer in number. *Thecopsora* resembles *Milesia* very closely, though the initial is somewhat sac-like with a narrowed base. In the subepidermal species, as in *Pucciniastrum*, they arise by division of the primordial cells, which cut off a row of vertical cells, the terminal ones becoming the initials while the lower ones lose their contents. These empty basal cells are most striking in *P. abietichamaenerii* and form a decided contrast to the compact group of initials just above them. The formation of teliospore initials in *Uredinopsis* takes place simply by the rounding up of the primordial cells.

The penetration of the host cell wall by the primordial cell and the haustorial mother cell might be briefly compared. *Calyptospora* is very suitable for such a study since early stages in the development of the initials were found in abundance. The primordial cell and the haustorial mother cell are similar in many ways although the former is much larger and its function is considerably different. According to Rice (19), in haustorial formation the wall becomes pierced and a small but definite papilla appears projecting slightly beyond the inner wall. The haustorium proper is formed at the end of this papilla. Moss (16) has some 30 figures of haustoria of the Pucciniastreae and this beak-like part, which extends through the cell wall, is a characteristic feature. In the penetration by the primordial cell there is a definite opening in the wall but papillae have not been found. In *Calyptospora*, as soon as the wall is pierced, the contents forms a small rounded sac-like body, the teliospore initial, which reveals no suggestion of a rod-like penetration tube. In the material studied by the writer there was never any difficulty in distinguishing the young initials from haustoria. Penetration of the cell wall undoubtedly takes place in the same manner as in the formation of haustoria. In their function and further development, however, the teliospore initials are radically different from haustoria.

On the basis of development, the species that have been studied fall readily into three general groups or types. In the first group the method of development is very simple. The primordial cells round up to form the teliospore initials, which lay down septations to form the teliospores directly. This is well illustrated by the developmental situation as found in *Uredinopsis*. The point of interest here is that *Uredinopsis* is generally considered to be the most primitive rust, and it also possesses a simple type of teliospore development. If a simple type of development may be regarded as a primitive character, then it is quite possible that *Uredinopsis* is the most primitive genus in the Pucciniastreae.

The second method of development is one where the primordial cells occupy the same relative position, but instead of becoming initials directly the host wall above becomes pierced, and the initials are formed *inside* the epidermal cell. The contents passes in and the initial enlarges to form the

intra-epidermal teliospore. This type of development is found in four genera, *Calyptospora*, *Milesia*, *Hyalopsora* and *Thecopsora*. In *Calyptospora goeppertiana* the teliospores are formed in the epidermal cells of the stems during the summer and completely fill every cell with thick-walled resting spores. *Hyalopsora aspidiotus* differs in that the spores are formed in the young fronds in the spring, and are thin walled and germinate immediately. In *Milesia marginalis*, *M. polypodophila* and *M. intermedia* the spores are similar to *H. aspidiotus*, but differ in that they are formed in the overwintered fronds in the spring. *Milesia fructuosa*, however, develops teliospores at the end of the growing season. *Thecopsora vacciniarum* differs from *M. fructuosa* only in that the teliospores overwinter and germinate in the spring.

In the third type the primordial cells tend to be aggregated in groups, and by division form a crust of initials which lay down anticlinal walls to form teliospores. This method of development is found in the genus *Pucciniastrum*. In *P. epilobii* the primordial cell usually divides once to give an empty basal cell, and an upper cell, which is the teliospore initial. This may be carried still further in *P. abieti-chamaenerii* where the primordial cell undergoes a number of divisions, cutting off a row of empty basal cells. The initial is greatly elongated, and as vertical septations are laid down the walls become greatly thickened. The teliospores thus become closely packed in groups, and may cover a considerable area.

These three general types seem to indicate two lines of development. With *Uredinopsis* as a starting point we have a subepidermal primordial cell which rounds up to form a teliospore initial, giving rise to a multicellular teliospore. Were the teliospore initial to be formed *inside* the epidermal cell instead of below, we would have the situation as now understood in *Milesia*, *Calyptospora*, *Thecopsora* and *Hyalopsora*. Thus, there seems to be a line of development culminating in the genera with intra-epidermal teliospores. The other line of development also begins with *Uredinopsis*, but leads through *Pucciniastrum* giving a compact subepidermal group. This is accomplished by the increase in number of the primordial cells and therefore in the number of teliospore initials. This line has tended more towards the sorus type of teliospore group. In *Melampsoridium betulinum* the teliospores are single celled and form small compact clusters, which arise from primordial cells, as in *Pucciniastrum*. In *Melampsora* there is said to be a definite sorus. The writer has studied early stages of *M. mcclusae* in a preliminary way and has found a group of primordial cells developing below the epidermis, as in *Pucciniastrum*, and in its manner of development it is similar to that of *Melampsoridium*. Hence, this series from *Uredinopsis* may lead through *Pucciniastrum* and *Melampsoridium* to *Melampsora*.

These results agree in a very general way with the phylogenetic tree that Faull (5) has constructed for the *Pucciniasreae*, except for the possibility that the two lines of development indicated above might represent two phylogenetic series. In that case, the phylogenetic tree would possess two main branches, both beginning at *Uredinopsis*, the one leading through the



intra-epidermal forms, *Milesia*, *Hyalopsora*, *Thecopsora* and *Calyptospora*, and the other passing through the subepidermal genera *Pucciniastrum* and *Melampsoridium*, to such forms as *Melampsora*. Many more data would be necessary, however, before this series could be postulated as a phylogenetic one.

These primitive rusts possess certain characters which they share with members of the Auriculariales and which point to their close relationship. Many authors have called attention to the fact that certain of the Auriculariales were rust-like and the similarity of the basidium and the promycelium has been often emphasized. It is quite generally conceded also that the probasidium and the teliospore are homologous structures (Gäumann (9), Linder (15)). It may be shown, moreover, that from the standpoint of their development there is a striking similarity. In *Uredinopsis* the teliospore has its origin in a terminal hyphal cell which has been called the primordial cell. The probasidium in *Iola*, *Saccoblastia* and in certain species of *Septobasidium* has its origin also in a hyphal cell, usually terminal in position, which is homologous with the primordial cell of *Uredinopsis*. It is significant also that in those forms in which the probasidium is not greatly differentiated, as in some species of *Septobasidium* and *Platyglea*, and also in such genera as *Eocronartium*, where the probasidium is entirely lacking, the origin of the basidium is in a hyphal tip, as in *Uredinopsis*.

The nuclear situation in the hyphal tip which gives rise to the probasidium in the Auriculariales is like that of the developing teliospore of the fern rusts. Two nuclei are always present and these come together and fuse as the probasidium matures. In *Iola*, Gäumann (8) found that as soon as the nuclei fused in the mature probasidium, germination took place and the fusion nucleus passed through all stages of prophase before entering into the basidium. A similar situation has been found in *Milesia* and *Hyalopsora* where the two nuclei fuse and immediately enter into heterotypic prophase before passing out into the promycelium. While very few of the Auriculariales have been studied cytologically it is interesting to note here that in *Eocronartium*, which has been described by Fitzpatrick (7), the details of nuclear fusion and meiotic prophase are practically identical with those of *Hyalopsora*. The fact that heterotypic prophase occurs in the teliospore and probasidium would lend support to the idea that the promycelium is an interpolated structure, since in the primitive condition not only prophase but anaphase and telophase took place in the spore itself.

This evidence is in line with the suggestion of Linder (15) that the Auriculariales have been derived from the Uredinales and not the reverse, as Neuhoﬀ (17) and Gäumann (9) believed. It is suggested here that the Auriculariales have arisen from some point on the basic rust line near *Uredinopsis*, *Milesia* and *Hyalopsora*, through such genera as *Iola* with a well-defined probasidium, *Septobasidium* which displays a gradual reduction in the probasidium, *Platyglea* where it has practically disappeared, to such forms as *Eocronartium* and *Auricularia*.

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# A COMPARISON OF VARIOUS HARVESTING METHODS IN RESPECT TO MOISTURE AND GRADE OF THE GRAIN<sup>1</sup>

(INTERIM REPORT)

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## Abstract

In a moisture and grade survey of grain harvested by various methods in western Canada during the 1932-1933 season it was found that straight-combined wheat showed a greater percentage of tough and damp samples than either stook-threshed or swath-combined samples. Of 401 stook-threshed samples 3% were tough or damp; of 416 straight-combined samples 22% were tough and 3% damp; and of 211 swath-combined samples 8% were tough and 1% damp.

With respect to grade, it was found that as a result of exposure to rains the average grade lowering was least for stooked grain and greatest for swath-combined grain.

Since the introduction of the combine-harvester into western Canada, statements have been made occasionally to the effect that combine-threshed wheat, especially that harvested by the straight-combine, is more liable to damage by heating than grain threshed from the stook. This has been attributed to (1) the tendency of operators to cut the grain containing too much moisture; (2) the presence of green weed seeds; (3) the presence of green kernels in grain from fields that have matured unevenly; (4) the failure of the grain to undergo the so-called "sweating-process" before it goes to the bin.

It seems likely that in some cases the farmer may begin combining too soon. The temptation to do so is great because the standing grain is subject to damage by wind, rain, hail and snow. Furthermore wheat will handle nicely in the combine several days before it gets below 14.4% moisture. The mistake of starting too soon, although made in the early days of use of this machine, is not now as common with experienced operators. Probably this factor will always have to be watched closely by buyers.

The presence of green kernels owing to uneven ripening is a matter for serious concern in some seasons, and in rolling country will always be a difficulty in the way of successful combining. The machine operator, faced with the alternative of going around or through a low spot containing a little immature grain, will usually take the latter course, hoping that there will be enough dry grain and that there will be sufficient mixing to reduce the moisture content of the bulk to a safe level.

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Green weed seeds, particularly those of Russian thistle, are extremely difficult to separate in a small thresher. In dry seasons, such as 1931-1932, when this weed was particularly prevalent, much of the wheat was heavily contaminated. Many weeds are not nearly ripe at time of harvesting, and consequently the seeds and parts of the stems break off and go into the wheat. The moisture content of such seeds may run as high as 60% and on account of their softness they tend to stick together in masses, which create centres of heating. This trouble is avoided in stook-threshing because the weeds, after cutting, desiccate fairly rapidly in normal weather, and the dry seeds even though they get into the grain are not a source of danger.

The sweating process is probably due to colloidal changes. It appears that the moisture which apparently increases in the outer layers of the kernels does not represent a loss or gain for the whole bulk, but is merely a matter of changed distribution. If this is the correct explanation there appears to be no reason why wheat should not undergo the change in the head as well as in the stook, but the general opinion of farmers and buyers is that this is not the case. It is thought that unless wheat sweats in the stook, it will sweat in the bin, and in the latter event will heat as a result of the moisture apparently produced in the process. Some grain men think that the difficulty of judging moisture of straight-combined grain is due to the fact that it has not sweated. Cases have been reported of grain reputed to be sound and dry going out of condition in transit.

These questions cannot be settled satisfactorily in the laboratory, and therefore it was decided that a survey of harvesting methods should be carried out in order to ascertain to what extent the various statements and beliefs previously mentioned, are warranted. To this end, during the harvest season

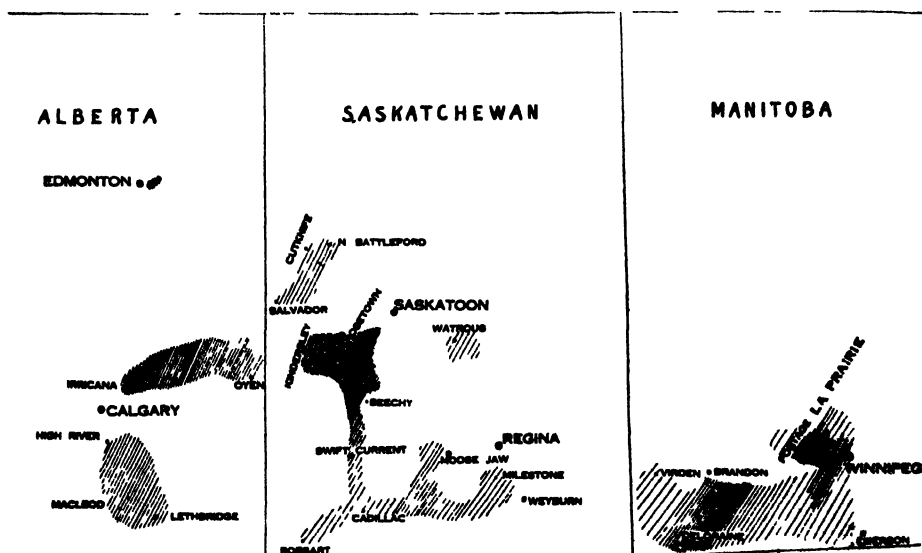


FIG. 1. Map showing roughly the areas in which samples were collected in 1932. Relative numbers indicated by intensity of shading.

of 1932, samples from machines in operation and from loads and bins of grain were collected by men sent out by the Universities of Manitoba, Saskatchewan and Alberta. The samples were taken in air-tight tins which were shipped each day to the respective laboratories where they were submitted to moisture determinations. Operations were confined to those areas in which a fairly large number of combines or swath-combines were operating. The districts covered in the three provinces are shown approximately by the map in Fig. 1.

The collections can be roughly divided into three parts: (1) the early harvest season, during which the weather was dry in all provinces and a large number of samples were collected under conditions in which the results were largely a reflection of farmers' judgments as to the proper time to begin operations; these data are especially valuable in connection with straight combining; (2) a short period during which harvesting operations were resumed for about two to three days following a fairly heavy rain; and (3) the remainder of the harvest season, after two or more rains had occurred. The numbers of samples collected in these periods in each of the provinces are given in Table I.

TABLE I  
CLASSIFICATION OF SAMPLES ACCORDING TO METHOD OF HARVESTING, EXPOSURE  
TO RAINFALL AND ORIGIN

	Before rain		After one rain		After two or more rains		Grand total	
	No.	%	No.	%	No.	%	No.	%
<b>Stook-threshed</b>								
Manitoba	136	13.2	13	1.3	61	5.9	210	20.4
Saskatchewan	65	6.3	8	0.7	62	6.1	135	13.1
Alberta	3	0.3	2	0.2	51	5.0	56	5.5
Total	204	19.8	23	2.2	174	17.0	401	39.0
<b>Straight-combined</b>								
Manitoba	39	3.8	13	1.3	19	1.8	71	6.9
Saskatchewan	57	5.5	13	1.3	175	17.0	245	23.8
Alberta	26	2.5	21	2.0	53	5.2	100	9.7
Total	122	11.8	47	4.6	247	24.0	416	40.4
<b>Swath-combined</b>								
Manitoba	103	10.0	4	0.4	44	4.3	151	14.7
Saskatchewan	9	1.0	2	0.2	23	2.2	34	3.3
Alberta	0	0	2	0.2	24	2.3	26	2.6
Total	112	11.0	8	0.8	91	8.8	211	20.6
Grand totals All methods	438	42.6	78	7.6	512	49.8	1028	

The total number of samples collected was 1,028; of these 39% were stook-threshed, 40% straight-combined, and 21% swath-combined; with respect to weather, 42.5% were collected before any rain, 7.5% after one rain, and 50% after two or more rains.

In a study like this, the two important considerations are the moisture content and the grade. For the sake of simplicity in the tabulations, we shall deal with these separately. In summarizing the moisture data, the durum wheat samples have been included with the common, because with respect to moisture the class of wheat should exercise no marked effect.

### **Moisture in Wheat Samples Harvested before Rain**

A summary of the distribution of moisture in this class is given in Table II. In this group there should appear evidences of the total effect of (a) the tendency to start combining too early, (b) unevenness in ripening, (c) green weed seeds if present to any appreciable extent. With both stook-threshed and swath-combined methods the samples showing moisture contents below 14.5% represented 98% of their respective totals, while with the straight-combined method, 12% were tough and 4% damp. The number in each group is sufficiently large to make this difference fairly significant, and it must be concluded therefore that in the early part of the harvest season, the tendency for straight-combined wheat to be tough or damp is appreciably greater than for stook-threshed or swath-combined grain. It should be noted too that the average moisture content of the straight-grade samples was higher for the straight-combined wheat than for the others. From the stook and swath there were 32 and 45% of the samples respectively that showed moisture below 10.9%, while only 3% of the straight combined were below this value. In fact, in the 416 samples of straight-combined grain, only 19 or 4.6% had a lower moisture content. This of course is a consequence of the fact that when standing grain gets to this moisture level it is too ripe, and is subject to heavy loss by shelling, and farmers therefore try to harvest just as soon as possible after the grain dries to below 14.4% or what they judge to be "straight-grade" moisture.

### **Samples Harvested after One Rain**

This group, though small, comprising only 77 samples or 7.5% of the whole series, is very interesting because the rain was heavy enough to stop all harvesting operations in the districts in which the writers were interested. It was followed by good drying weather which lasted just long enough to permit a partial resumption of operations. While it is obviously impossible in a summary such as this to examine the circumstances of each particular case, it would appear probable that this group includes samples from the more impatient operators and from those having the poorer judgment regarding the fitness of wheat for threshing. Examination of Table III shows that only three out of 23, or 13% of the stook samples, were tough; 14 out of 46, or 30% of the straight-combined samples, were tough, and 2% of them were damp, while six of the eight swath samples were tough. These data may be interpreted as evidence either that the combine operators were too anxious to begin or that their judgment was poor.



**TABLE III**  
**DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES CUT AFTER ONE RAIN**

Moisture range %	Stook-threshed						Straight-combined						Swath-combined					
	Man.	Sask.	Alta.	Total	%	Av. moist- ure	Man.	Sask.	Alta.	Total	%	Av. moist- ure	Man.	Sask.	Alta.	Total	%	Av. moist- ure
Straight < 10.9 10.9-11.7 11.8-12.6 12.7-13.5 13.6-14.4	—	—	1	1	4	10.2	—	—	3	3	6	10.5	—	—	—	—	—	—
	—	—	1	1	4	11.3	—	—	3	3	6	11.3	1	—	—	1	12.5	11.2
	4	—	—	4	18	12.4	1	1	5	7	15	12.2	—	—	—	—	—	—
	6	2	—	8	35	13.1	2	1	3	6	13	13.1	—	—	1	1	12.5	13.2
	3	3	—	6	26	14.1	5	5	2	12	26	14.0	—	—	—	—	—	—
Tough 14.5-15.3 15.4-16.2 16.3-17.0	—	—	—	—	—	—	5	2	—	7	15	14.9	2	1	1	4	50	15.0
	—	2	—	2	9	15.9	—	1	—	1	2	15.7	1	1	—	2	25	15.6
	—	1	—	1	4	16.5	—	2	4	6	13	16.6	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Damp 17.1-17.9 18.0-18.8 18.9-19.7 > 19.7	—	—	—	—	—	—	—	—	1	1	2	17.4	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	1	—	1	2	19.2	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total in each group						47						8						
Total series, 78 samples																		



### **Samples Collected after Two or More Rains**

Table IV shows a summary of the samples collected after the second and subsequent rains. It should be observed here that this occurrence of two heavy rains in rather close succession had the effect of postponing harvesting operations in the combine area of Saskatchewan and Alberta for two to three weeks after the grain had become fit for harvesting. The most important consequence of this was that one of the reputed sources of trouble, namely, unevenness of ripening, was almost wholly removed because, during the period required for the standing grain to become dry enough to cut, the slower maturing heads had a chance to ripen. This partly accounts for the evenness and high grade of the combined samples of this season.

Furthermore, owing to better climatic conditions in the growing period, wheat had a vigorous growth and the Russian thistle was less prevalent than in the previous year. Other weeds ripened during the two to three week delay previously mentioned and hence combined wheat was in nearly all cases almost free from green weed seeds. The incidence of tough and damp samples shown in Table IV therefore must be attributed largely to the too early resumption of operations and again, this was due to anxiety, poor judgment, or both.

In this group there were 247 straight-combined samples, of which 3% were damp, 25% tough and 72% straight grade; 174 stook-threshed samples of which 3% were tough and 97% straight; and 91 swath samples of which 2% were damp, 9% tough and 89% straight grade. Most of the straight-combined samples in this group were collected in Saskatchewan. After the rains of August 29 to 31, the weather conditions were quite favorable for harvesting. There were only slight local showers from then until the end of the collection period. It must be concluded therefore that there may be, even under the most favorable conditions, a relatively high percentage of straight-combined wheat cut in the tough stage.

Part of the trouble, particularly in the latter part of the harvest season, seemed to be due to the fact that the straw, having become practically dead, dries out fairly rapidly when standing, and the farmer, accustomed to judging the fitness of grain by the condition of the straw, starts his machine when the straw is brittle enough to thresh properly. This criterion is generally satisfactory for stooked grain because the heads, being more open and exposed, dry more rapidly after a heavy rain than the more closely packed straw, and thus, when the straw is fit for threshing, the grain usually is also fit. This does not hold in the case of standing grain. The writers were able to procure a small series of samples under conditions that show this very clearly.

On September 9 about midnight, the weather in the Rosetown area suddenly changed from hot and dry to cool and cloudy, and a very slight amount of rain, too small to be recorded except as a trace, fell. This was followed by a very heavy mist which lasted until about 7 a.m. September 10. The weather remained cool and cloudy until 10 a.m. when it cleared and became warmer. Stook threshing was resumed at about 3 p.m., but the combines



stood idle until about 6 p.m. Samples of stook, combine and swath grain were collected in order to ascertain the effect of this particular weather condition. The results, given in Table V, show that of 17 stook samples only 6% were tough; of 27 combine samples 37% were tough and 7% damp; of 15 swath samples, 26% were tough and none damp. The strange thing

TABLE V

SAMPLES COLLECTED ON SEPTEMBER 10, 3 TO 9 P.M., AFTER A VERY SLIGHT SPRINKLE OF RAIN FOLLOWED BY MIST AND CLOUDY WEATHER

Moisture range %	Stook			Combine			Swath		
	No.	%	Average moisture	No.	%	Average moisture	No.	%	Average moisture
10.9-11.7	1	6	10.9	2	7.4	11.1	—	—	—
11.8-12.6	6	35	12.4	1	3.7	11.8	5	33.3	12.3
12.7-13.5	8	47	13.0	4	15.0	13.2	4	24.6	12.9
13.6-14.4	1	6	13.8	8	29.6	13.9	2	12.3	14.1
<b>Tough</b>									
14.5-15.3	1	6	14.6	6	22.2	14.9	1	6.7	14.8
15.4-16.2	—	—	—	2	7.4	15.8	1	6.7	15.9
16.3-17.0	—	—	—	2	7.4	16.5	2	12.3	16.7
<b>Damp</b>									
17.1-17.9	—	—	—	1	3.7	17.1	—	—	—
18.0-18.8	—	—	—	1	3.7	18.2	—	—	—
<b>Totals</b>	17			27			15		

about this was that the stooks appeared to be on the tough side, while the standing grain in all cases was brittle and in good threshing condition. In the case of the stooks, the inside of the sheaves was very dry and even though the outer heads were quite tough, they formed so small a percentage of the whole that the representative sample taken from the load was quite dry. The combine grain was uniformly higher in moisture and the lag of drying in the head, probably owing to protection by the glumes, resulted in a tough sample.

A general summary of all the moisture data is given in Table VI. This includes Tables II, III and IV, Table V having already been included in Table IV. Of the 401 stook-threshed samples only 3% were tough and none were damp; of the 416 straight-combined samples 22% were tough and 3% damp; and of 211 swathed samples, 8% were tough and 1% damp. It may be concluded from these data that there is a much greater tendency for straight-combined wheat to run tough or damp than grain harvested by other methods. The prevailing harvest weather, despite the rains that occurred in the earlier part, was on the whole, very favorable. If under such circumstances 25% of the samples were tough or damp, it might be expected that in an unfavorable season, the percentage would be larger.

TABLE VI  
DISTRIBUTION OF MOISTURE, ALL WHEAT SAMPLES COLLECTED

Moisture range %	Stook-threshed				Straight-combined				Swath-combined			
	Man.	Sask.	Alta.	Total	%	Average	Man.	Sask.	Alta.	Total	%	Average
Straight grade												
< 10.9	59	13	17	89	22.2	10.2	—	8	11	19	4.6	10.5
10.9-11.7	56	30	15	101	25.2	11.3	19	34	16	69	16.6	11.3
11.8-12.6	45	39	13	97	24.2	12.3	11	41	26	78	18.7	12.1
12.7-13.5	29	33	7	69	17.2	13.1	9	44	17	70	16.8	13.0
13.6-14.4	17	13	3	33	8.2	14.1	17	47	12	76	18.3	14.0
Totals				389	97.0					312	75.0	
Tough												
14.5-15.3	—	4	1	5	1.2	14.7	9	37	6	52	12.5	14.9
15.4-16.2	3	2	—	5	1.2	15.6	4	19	2	25	6.0	15.8
16.3-17.0	1	1	—	2	0.5	16.5	—	7	8	15	3.5	16.6
Totals				12	3.0					92	22.0	
Damp												
17.1-17.9	—	—	—	—	—	—	1	2	2	5	1.2	17.4
18.0-18.8	—	—	—	—	—	—	1	4	—	5	1.2	18.3
18.9-19.7	—	—	—	—	—	—	—	1	—	1	0.3	19.2
19.8-20.6	—	—	—	—	—	—	—	—	—	—	—	—
> 20.6	—	—	—	—	—	—	—	1	—	1	0.3	20.6
Totals										12	3.0	
Grand totals	401				416				211			
Total series, 1,028 samples												

In concluding the discussion of moisture, it should be noted, as a matter of record, that of the 71 tough and damp samples in the Saskatchewan group, only one was graded into the local elevator as tough, that one being the sample with 20.6% moisture. In all other cases, the buyers took the grain in as straight grade, and as there was a great proportion of cash sales, loss could easily be avoided by mixing with drier grain of the same grade. The buyers in areas where a great deal of straight combining is done, professed to have no apprehension about handling it. In these districts the farmers are guided to a large extent by the judgment of the buyer; they take in a batch from the first round of the machine and get the buyer's opinion regarding its fitness. If the buyer thinks the grain a little too tough, he advises the operator to wait for a day or two, and usually this advice is acted upon. On the other hand, in districts where few combines are used, there is often a reciprocal distrust between farmer and buyer, the farmer suspecting unwarranted bias on the part of the buyer, who in turn is inclined to discount combined grain unduly because he has not handled enough to have learned the limits of safety. This attitude is somewhat justified, because generally the districts having few combines operating are those to which they have proved unsuitable owing to rolling topography, or late ripening period. Briefly then, the buyers who condemned most vigorously the straight combine were those who had the least of this kind of grain to handle. In the Saskatchewan survey there were encountered very few buyers who admitted having lost cars from heating.

### **The Relation of Harvesting Method to the Grade of the Sample**

The season of 1932 was rather unsuitable for a study of grade because, owing to factors already discussed, the crop was very uniform, practically none of it grading below No. 3 Northern, and by far the largest percentage grading No. 1 Hard or No. 1 Northern. This narrows down the range and makes differentiation on this basis more difficult than if there had been a wider grading range.

Only the Manitoba and Alberta samples were officially graded, and the results from these provinces have been collected in Tables VII and VIII, the former showing the division according to weather and the latter showing a summary of the whole series. The durum samples have been treated separately in Table IX.

Considering Table VII, it can be seen that in the collections made before any rain, the percentage of samples in the four grades was nearly the same for both stook- and straight-combined samples, being 32-35% No. 1 Hard, 47% No. 1 Northern, 13-14% No. 2 Northern and 4% No. 3 Northern (the combined samples are quoted first). The swathed samples showed only the top three grades, No. 1 Hard and No. 1 Northern each representing 46.5% of the total number; there were thus more No. 1 Hard samples by this method than by either of the other two.





TABLE IX  
SUMMARY SHOWING THE MOISTURE CONTENT FOR GRAIN HARVESTED BY THE VARIOUS METHODS

Method of harvesting	No. of samples collected	% of samples grading			Moisture			% of samples grading				Average grade
		Straight	Tough	Damp	Min. %	Max. %	Av. %	1 A.D.	2 A.D.	3 A.D.	4 A.D.	
Stook-threshed												
Before rain	44	100.0	—	—	10.5	13.9	11.6	97.7	2.3	—	—	1.02
After 1 rain	10	100.0	—	—	12.3	14.0	13.2	90.0	10.0	—	—	1.10
After 2 or more rains	40	97.5	2.5	—	10.6	15.3	12.7	60.0	37.5	—	2.5	1.55
								Mean for stook-threshed				1.25
Swathed and combined												
Before rain	60	96.7	1.7	1.7	10.3	14.0	11.5	98.3	1.7	—	—	1.01
After 1 rain	4	25.0	75.0	—	11.2	15.4	14.2	50.0	25.0	25.0	—	1.80
After 2 or more rains	31	87.1	9.7	3.2	10.6	14.9	13.4	19.4	48.4	32.2	—	2.10
								Mean for swath-combined				1.40
Straight-combined												
Before rain	18	88.9	5.6	5.6	11.5	17.3	13.1	94.4	—	5.6	—	1.11
After 1 rain	11	63.6	36.4	—	12.5	15.0	14.1	100.0	—	—	—	1.00
After 2 or more rains	17	70.6	23.5	5.9	11.3	18.4	13.5	58.8	29.4	11.8	—	1.50
								Mean for straight-combined				1.23



The group representing those collected after one rain is too small to give reliable results, but it is evident, in the straight-combined samples, that this exposure to rain had the general effect of increasing the number of No. 1 Northern samples at the expense of the No. 1 Hard group.

The exposure to two or more rains shows this tendency still more and in this group only 9% of the combined samples graded No. 1 Hard, as compared with 32% for the first group. Forty per cent of them graded No. 2 Northern as compared with 13% in the "before rain" group. The stook samples exhibit this tendency to a much lesser extent. The swathed samples, after two rains, showed a sharp decrease in percentage of No. 1 Hard and No. 1 Northern grades, with a corresponding increase in No. 2 Northern and the rejected grades, 24% falling into the latter category. This is owing to sprouting and moulding which occurred when the cut grain had been beaten down too close to the ground by rain. The rains during the collection period were not of sufficient extent or duration to effect any of this type of damage to either stooked or standing grain. These data merely confirm the general observation that grain in the swath is more susceptible to damage by moderate rain than grain in any other condition.

The change in value, from the grade standpoint, can be seen more readily by computing a sort of mean or average grade for the various methods and periods. In calculating such a figure it was necessary to assign arbitrary numerical values to the inspector's grades. For example, in the instance of common wheat, the values assigned to No. 1 Hard, Nos. 1, 2, 3 and 4 Northern, No. 5, No. 1 Northern rejected, No. 2 Northern rejected, No. 3 Northern rejected and No. 4 Northern rejected were 0, 1, 2, 3, 4, 5, 3, 4, 5, and 6, respectively. That is, the rejected grades were all dropped two units, which corresponds roughly to the average price discount.

The average grades calculated on this basis show that, while samples by all methods graded lower after two or more rains, the lowering was least in the stook and most in the swath, with the standing grain occupying an intermediate position. Considering all samples by the various methods, grouped without reference to period of collection, as in Table VIII, the average grades for stook, straight-combine and swathed samples are 1.04, 1.35 and 1.40, respectively. If these samples can be considered representative of the season's crop, it may be concluded that with both the straight-combine and the swathed combine, the average loss due to grade lowering compared to stook threshed grain, could be represented by a value equivalent to one third of the spread between No. 1 Northern and No. 2 Northern.

The durum wheat samples which were collected only in Manitoba showed somewhat different results. As can be seen from Table IX, the effect of exposure to two or more rains was about the same on both stook and straight-combine samples, and the general grade average for each of these classes is practically the same, being 1.25 and 1.23, respectively. The swath samples showed evidence of heavier grade damage even after exposure to one rain, the loss being about one grade. While the weighted mean given in the table

shows a value of 1.40 for average grade of all the swath-combined samples, the disproportion in numbers collected before rain and after two or more rains, doubtless biases this value toward the lower side. However, it may be concluded that the stook and straight-combine samples lost the equivalent of one-half grade, while the swath samples lost about one full grade as a result of exposure to several rains.

Summarizing the grade study, we may state that the stook-threshed samples showed least damage from rains, the swath-combine samples most, and on the whole the straight-combined samples were intermediate between these two.

### Conclusion

The moisture and grade survey conducted in the 1932 harvest period has been very enlightening, inasmuch as, in a season as favorable as this, when practically all grain, no matter how harvested, was going forward as straight grade, it was found that 25% of the 416 samples of straight-combined wheat were actually tough or damp. This condition existed despite the fact that most of the factors complicating the harvesting of grain by this method were practically absent, when the bulk of the grain was being cut. Reference is made particularly to green weeds, uneven ripening and protracted rainy weather. Most of the fault in the season under study must, therefore, be attributed to the judgment of the operators. It appears reasonable to expect that in an unfavorable season, the percentage of tough and damp samples would be much larger.

# NOTES ON THE BIOLOGY OF CERTAIN TORTRICID SPECIES WITH STRUCTURAL DETAILS OF THE LARVAE AND PUPAE<sup>1</sup>

By J. McDUNNOUGH<sup>2</sup>

## Abstract

Biological notes are given on six species of Tortricidae—*Sparganothis directana* Wlk.; *Tortrix alleniana* Fern.; *Cacoecia myricana* McD.; *Cacoecia parallela* Rob.; *Tortricodes horaria*ana Wlsh. and *Peronea cornana* McD.; three species of Eucosminae—*Epinotia lindana* Fern.; *Epinotia myricana* McD. and *Anchylopera semiovana* Zell.; five species of Argyroplocinae—*Exartema cornanum* Heinr.; *Exartema permundanum* Clem.; *Exartema valdanum* McD.; *Argyroplce albiciliana* Fern. and *Evora hemidesma* Zell.

A comparative study of the setal pattern of both head and abdomen in the larvae of the above-mentioned species is presented and the main structural details of the pupae are also described. In this connection a critical comment on both Fracker's and Mosher's generic keys, based respectively on larval and pupal characters, is offered.

In the course of breeding work carried on in the Bobcaygeon region in Ontario during the summers of 1931 and 1932 the writer accumulated in alcohol a small collection of definitely associated and determined larvae of Tortricid species (in the broad sense), belonging to a number of scattered genera. Several of these fall into the family Tortricidae, the others into the subfamilies Argyroplocinae (Olethreutinae) and Eucosminae of the Eucosmidae, as treated by Heinrich (9, 10). As the life histories and food plants of the above-mentioned species are for the most part unknown, the writer offers in the present paper some biological details taken from field notes, and supplements these with structural studies of the individual larvae, these studies being largely based on the keys given in the Tortricid section of Fracker's paper (7) on the Classification of Lepidopterous Larvae, which has been reprinted by Forbes (6, p. 385) with certain generic changes.

With very limited material for study at his disposal Fracker was unable to offer at the time either subfamily or generic keys which would bring a larval classification into line with the system of classification then in use and based on adult characters; this system, as far as the Eucosmidae are concerned, has been since considerably altered by Heinrich's revision and the generic concepts in many instances radically changed. The present material has, of course, been far too scanty to do more than act as a sort of check on Fracker's work and the characters used for generic separation; in the main these characters have been found reliable, but in some cases where the generic value was doubtful this has been indicated under the individual species.

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A few brief notes on the various pupae have been appended, but a detailed study of them has not been made and will have to be left for a future occasion. The writer has confined himself mainly to enumerating structural points which are contradictory to the keys given by Mosher (12).

A great deal more work on the individual species will have to be done before any really satisfactory generic keys can be tabulated, but, as Fracker states, the larvae are of sufficient economic importance to warrant the expenditure of considerable time in such study, and the writer hopes to continue work along similar lines as occasion permits.

### Tortricidae

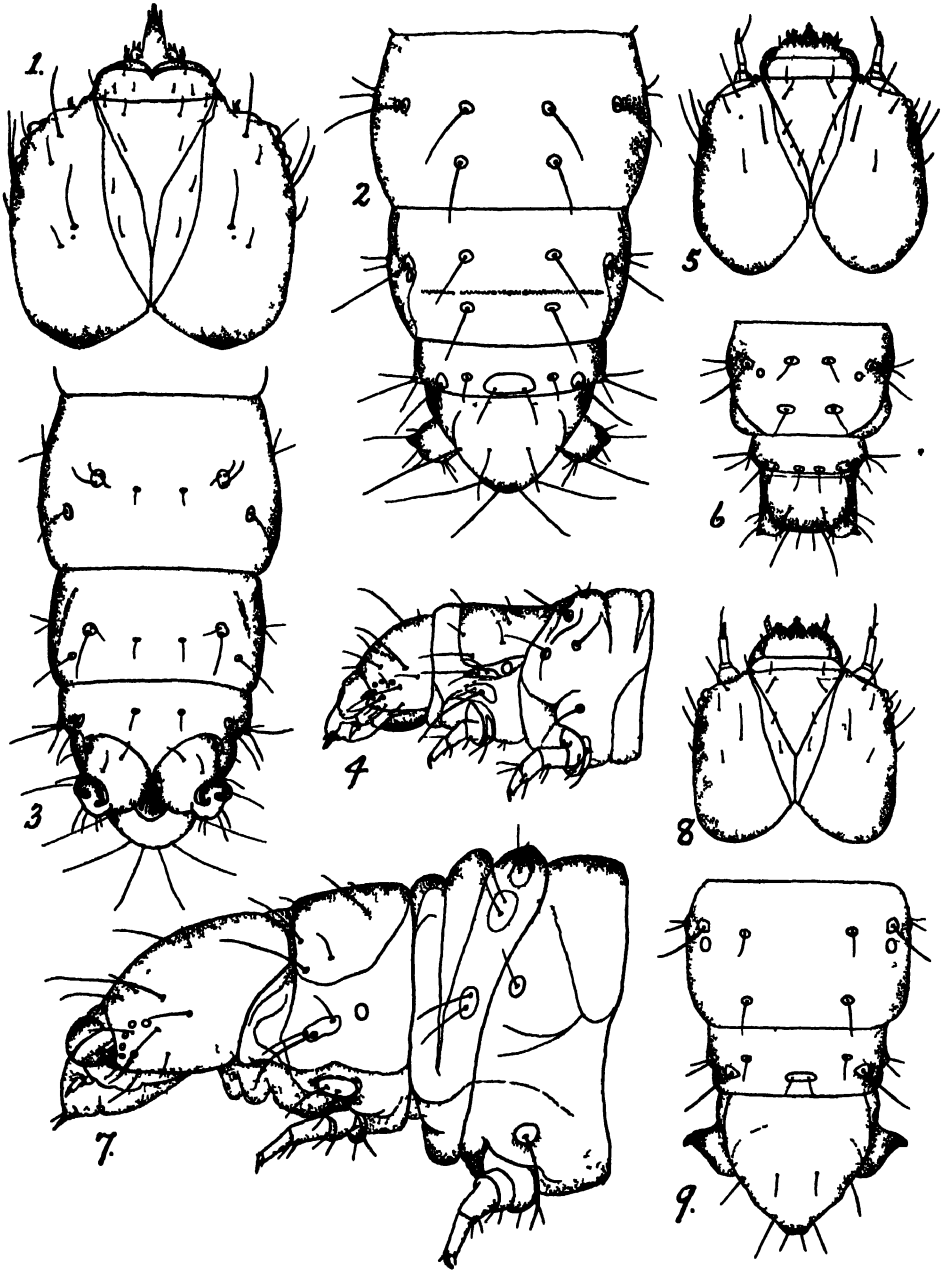
#### *Sparganothis (Cenopsis) directana* Wlk.

This species has been classified in current check lists as synonymous with *reticulatana* Clem. Dr. Dyar, however, as early as 1902 (2, p. 402) maintained that it was a distinct species and recorded the larva on wild cherry in Colorado; Fracker probably examined these very larvae as he records *Cenopsis directana* (7, p. 74) as one of the species studied.

The writer believes that Dr. Dyar was entirely correct in the above procedure, and Mr. A. Busck, with whom the writer has been in correspondence, confirms this determination. From a number of larvae found tying the terminal leaves of *Prunus virginiana* a series of moths was bred in late June, the males of which correspond quite closely to Walsingham's figure of the type of *directana* (14, p. 17); they show the same golden-yellow color on the primaries but the writer's specimens do not possess the complete, oblique, purplish median band shown in the figure, this being broken up into costal and dorsal spots, a variation frequently found in the entire group. The females have much darker forewings than the males with the purple-brown costal patches obsolescent; the light red-brown color of the secondaries in both sexes is quite characteristic.

Mature larvae have the head, prothoracic shield, pinacula of the prothorax and the legs deep blackish, the latter with the two terminal joints shading into paler brown. The mouth parts project rather strongly beyond the margin of the head capsule but the antennae are quite short (partly due to retraction in the material, in the writer's opinion). The body is entirely dark greenish dorsally as far laterad as the spiracular area and very faintly and finely shagreened; laterally and ventrally the color is a pale dull green, paler in color than the surrounding area; the pinacula are dull whitish but not strongly contrasting. The anal plate is conical, with convex basal margin, the anal fork is well developed and six- to eight-pronged.

As typifying the true Tortricids Figs. 1-4 are given showing the arrangement of the setae on head, thorax and abdomen. Particular attention should be given the characteristic position of *alpha*, *beta* and *rho* on abdominal segment IX, for in dealing with subsequent species attention will be merely called to any divergences from the figured arrangement shown here. A study of the figures will show that *directana* fits in excellently with Fracker's



FIGS. 1-4. *Sparganothis (Cenopsis) directana* Wlk. FIG. 1. Head (viewed dorsally). FIG. 2. Abdominal segments VII-X (viewed dorsally). FIG. 3. Abdominal segments VII-X (viewed ventrally). FIG. 4. Head and first two thoracic segments (viewed laterally). FIGS. 5-7. *Epnotoia lindana* Fern. FIG. 5. Head (viewed dorsally). FIG. 6. Abdominal segments VIII-X (viewed dorsally). FIG. 7. Head and first two thoracic segments (viewed laterally and much enlarged). FIGS. 8 AND 9. *Exartema cornanum* Heinr. FIG. 8. Head (viewed dorsally). FIG. 9. Abdominal segments VIII-X (viewed dorsally).

keys (7, p. 73) if we omit his captions *d* and *dd*. The position of the middle seta of the *Kappa* group on the prothorax in relation to the other two hardly bears out Fracker's contention that it is three times as far from the caudal seta as from the cephalic one; to the writer it appears to be only very slightly more than twice as far.

The pupa is rather dark brown; dorsally abdominal segments II–VIII show both rows of transverse blackish spines, although the caudal row on segment VIII is much reduced; there are no spines on IX or X. The cremaster is well developed, longer than broad, with four terminal and two lateral setae, making eight in all. There is a single laterocaudal seta on the anal rise, a feature which rather upsets Mosher's keys to families. The front shows a broad raised dumb-bell shaped area with smooth upper surface, somewhat constricted opposite the antennal bases.

A single female pupa of *Sparganothis unifasciana* Clem. from a larva feeding in a terminal shoot of a *Solidago* sp. shows a number of divergent features. While the frontal elevation and the spining of the first eight abdominal segments are similar, there is a very strong caudal row of spines on segment IX. The cremaster is greatly reduced, being broader than long and with the setae reduced to very minute spines of which two terminal ones are best detected. There is no seta on the anal rise.

#### *Tortrix alleniana* Fern.

This well-known species is recorded by Forbes (6, p. 492) as being a general feeder in the larval state, but the writer has met with no detailed account of the early stages apart from that of Venables (13).

A few mature larvae tying the terminal shoots of *Monarda fistulosa* in early June were secured and these produced adults (1 ♂, 2 ♀) on June 21, 28 and July 1. In the mature larva the head is pale ruddy, the epicrania faintly marbled laterally with light brown; the sides of the vertical triangle are broadly suffused with black-brown, this color extending forward along the adfrontal suture as far as the posterior seta and forming roughly a triangular patch; the posterior edge of the epicrania as far as the lateral tubercle is narrowly brown; a small spot anterior to the first posterodorsal seta, the region of the ocelli and the posterior margin of the clypeus are also dark brown. The antennae are short and the whole shape of the head and position of setae very similar to that of *directana* except that the posterior epicranial setae with their puncture form more nearly a straight oblique line, with the puncture closer to the second seta. The prothoracic plate is similar in color to the head with a large posterior dark brown median patch and a similarly colored, irregularly lunate, lateral patch. There is also a small brown spot on the prothoracic segment above and slightly posterior to the *Pi* group of setae. The legs are shaded outwardly with brown and have dark streaks anteriorly at their base. The entire dorsal surface of the abdomen is a dark, dirty green, the integument in this area being very finely shagreened and containing minute scattered rather regular-placed, pale lenticles, principally along the first transverse fold of each abdominal segment (close to anterior

margin) and in two groups of three or four above tubercle *rho* (III); the pinacula stand out strongly as pale circular areas; laterally the color is pale green, becoming deeper again on the ventral surface of all segments but the thoracic ones. The anal plate is pale, conical, broad at the convex base, the sides narrowing rather abruptly to a bluntly truncate apex; there is slight brown sprinkling, principally in the basal section. The anal fork is strongly developed with seven prongs, much as in *directana*. The spiracles are broadly oval, almost circular, brown, ringed with black. The setal arrangement appears to be exactly as in *directana*.

The deep black-brown pupa shows both rows of dorsal transverse spines on abdominal segments II–VIII, considerably reduced in size on II; deep transverse grooves are situated dorsally on anterior margins of II and III; there are no spines on IX and X nor setae on the anal rise. The cremaster is longer than broad with four terminal and two lateral setae. There is a slight raised anchor-shaped prominence on the front between the antennal bases.

*Cacoecia myricana* McD.

Larvae of this species were not very numerous but about eight specimens were taken on *Myrica* where they live in a sort of tube formed by joining several leaves loosely together; three larvae were also secured from an alder bush, growing alongside the *Myrica* which, on a superficial examination, could not be differentiated from the *Myrica* feeders. From the *Myrica* larvae the writer reared three males and three females, these adults emerging June 20–24; the three males agree quite well with the author's original type series from Algonquin Park, Ont.; two of the females also agree with the type females in having more or less unicolorous red-brown primaries with little evidence of maculation; the third female, however, is much paler and very similar to females of *eleagnana* McD. The single male bred from the alder feeders is more sepia-brown, rather than ruddy brown, in the color of the forewings and approaches therefore close to *mortuana* Kft. Finally from a mixed lot of unsorted larvae on *Myrica* the writer bred a single large female of typical *argyrospila*. The above results, it must be admitted, are not very satisfactory in determining the status of the various forms of the *argyrospila* group; either the larval series was mixed, owing to lack of equipment in the field to enable the detection of small differences, or else (what seems quite probable) we are dealing in the case of the *Myrica* feeders with a form which has not yet entirely established itself as distinct from typical *argyrospila*, but is certainly working in that direction. Breeding from eggs of a known female should clear up the situation to a certain extent.

The full-grown larva of the *Myrica* feeder (the only form of which material was brought back in alcohol for study) can apparently be distinguished from *argyrospila* larvae, as represented by material from Vineland, Ont. It has a pale reddish head with the labrum, mandibles, frontal sutures, patch around the ocelli, posterior edge of epicrania, and a lateral longitudinal stripe extending backwards from between the second and third ocellar setae, deep brownish; the posterodorsal portions of the epicrania are quite heavily marbled

with pale brown, leaving a broad vertical stripe of the ground color extending to posterior margin and containing the posterior epicranial setae. The epicranial lobes are rather more rounded laterally than in *directana* and the pale antennae are slightly longer, although still quite short. The most noticeable feature in the arrangement of the setae as compared with the figure of *directana* is that the two posterior epicranial ones with the almost equidistant puncture form a straight, only very slightly oblique, line; the lateral epicranial seta is less obliquely situated with regard to the third anterior one, the width between them is relatively greater and both are situated farther behind the ocelli than is the case in *directana*. The grouping of the ocelli seems to be practically similar and the writer cannot detect that the second ocellus is farther from the first than from the third one.

The prothoracic plate is pale with a small brown patch in the anterolateral corner and a faint brown lunate mark dorsad of tubercle *rho*. Dorsally the entire body is deep olive-green, paling into dirty whitish laterally; there are distinct traces of pale subdorsal longitudinal bands crossing the region of tubercles *alpha* and *beta* and particularly evident on the anterior portion of the abdominal segments. The pinacula are pale but not very contrastingly so and the spiracles are dull brownish with a distinct dark outer ring. The legs are deep brown at the base paling into light brownish on the two terminal segments. The anal plate is pale and very similar in shape to that of *directana*; it is sprinkled with small brown lenticles on the basal section; such lenticles occur very sparsely also on the abdominal segments, in sections similar to those of *alleniana*, but are not nearly as prominent as in this species. The anal fork is seven pronged, much as in *directana*, and the arrangement of abdominal setae is also similar to that of this species.

In comparison a typical *argyrospila* larva has a black-brown head and the entire lateral edge of the prothoracic plate is rather broadly suffused with brown. The body is pale green and shows none of the deep olive-green dorsal shading found in *myricana*. It is true that Dr. Dyar (2, p. 400) maintains that there is a great variability in color for the heads of *argyrospila* larvae, but this needs further proof before it can be accepted; it is possible that either Dr. Dyar had his series mixed or that he made his observations on larvae immediately after a skin-shedding when the pigmentation had not developed. On the other hand his description of the larva of *vividana*, which he considers to be merely a variety of *argyrospila*, would fit in quite well for typical *argyrospila*. Both Venables (13) and Criddle (1) note that the heads of *argyrospila* larvae are blackish.

The pupa is deep blackish-brown and seems considerably darker in color than that of a female *argyrospila* bred from *Viburnum* and also from a male pupa of the same species, bred from birch. The writer cannot, however, detect any structural differences. There is the usual double row of transverse spines dorsally on abdominal segments II–VIII, the cephalic row on II being greatly reduced; on IX and X there are no spines in the female and only a few minute spines on IX in the male. On the anterior margins of II and III



are two deep subdorsal pits, separated from each other by a narrow medio-dorsal ridge. There are no setae on the anal rise. The cremaster is longer than broad with four apical and two lateral setae. The front is not raised above the level of the antennal bases.

*Cacoecia parallela* Rob.

A few of the very striking larvae of this species were taken on *Myrica* along with those of *myricana*, the adults emerging at about the same time. With its reddish head and dark abdomen on which the white pinacula show up very distinctly, the larva is readily distinguished from other Tortricids feeding on the same plant.

The larva was briefly described by Edwards and Elliot (4, p. 80) and a rather full description was given by Fletcher (5), and further mentioned by Gibson and Ross (8, p. 18). The writer's observations correspond with those of the above authors and make it evident that the larva mentioned under this name by Washburn (15, p. 170) cannot have been correctly identified.

The head of a mature larva is light reddish with the mouth parts, antennae, a patch around the ocelli, a posterolateral longitudinal stripe on a level with the ocellar patch, the frontal sutures and posterior margin of epicrania, deep black-brown. There is some faint lateral light brown marbling on the epicrania and a trace of a dark streak on the lobes along the posterior section of the adfrontal suture. The shape of the head and the arrangement of the setae are practically as in *myricana*. The prothoracic plate is similar in color to the head with a broad black-brown border and a similarly colored antero-medial patch; the prothoracic pinacula and the legs are also black-brown.

With the exception of the lateral portion of the prothorax which is paler, the entire body is blackish-olive, the skin very finely shagreened and the white pinacula standing out very markedly from the dark body-color. There are scattered lenticles over the body surface as in *Tortrix alleniana* but not so prominent. The spiracles are pale brownish, ringed with black. The anal plate is somewhat paler than the body color and rather more bluntly conical than in *directana*, being almost as broad at its widest part as it is long. The anal fork is strongly developed, with six prongs. The setal arrangement of thorax and abdomen does not differ from that of *directana*.

Fracker includes this species in a section in which the *Pi* group on the meso- and metathorax is bisetose; the writer's material does not show this, on both segments the group being normal and unisetose.

The pupa is black-brown with the usual two transverse rows of dorsal spines on abdominal segments II-VIII, both rows somewhat reduced in size on II, the caudal row on VIII composed of very small spines in the male and both rows reduced in the female; a few minute spines on IX in the male and none in the female; no spines on X in either sex nor any setae on anal rise. Cremaster much longer than broad with four terminal and two lateral setae. Fairly deep transverse furrows on anterior dorsal margin of II and III. Front with strongly raised broad, transverse ridge between the antennal

bases, connected by a narrow ridge with a raised, inverted-U mark on the caudal section of the front. A very similar structure occurs in the pupa of *C. rosaceana* Harris, but in this species is less prominent.

*Tortricodes horariana* Wlsh. m.

Larvae of this species were not uncommon on wild gooseberry; they live singly, spinning up the terminal leaves into a small white web, somewhat like that of a Phycitid. They mature during early July and spin a tough, boat-shaped cocoon on the stalk of the plant, pupating within 10 days; emergence does not, however, take place until early fall, the dates on the writer's specimens ranging from September 20 to October 3.

The larva is entirely pale green. The head is rather flattened dorsally, almost as broad as long, with a distinct frontal suture, but with the adfrontal sutures scarcely distinguishable; the labrum is light brownish in color (alcohol specimen), not particularly large or prominent; the antennae are quite long and extend when fully drawn out to a level with the apex of the maxillary palpi. Setal pattern and other details much as in *directana*; the lateral epicranial seta (viewed from the side) is less obliquely situated with regard to the third anterior one and is more as in *Cacoecia myricana*.

Abdomen rather wrinkled, with large but improminent pinacula, due to the similarity in color to that of the abdomen; anal plate smooth, rather more than hemispherical, broadly rounded apically; anal fork well developed, five pronged. Spiracles small, pale, almost circular. Crotchets of prolegs biordinal. According to Fracker's generic key (7) the species would trace down to the same group as *Tortrix* and *Cenopsis*, if we disregard his caption *d* which calls for the middle seta of the trisetose *Kappa* group on the prothorax to be three times as far from the caudal seta as from the cephalic one, in contrast to *dd* where the distance is only twice as far. In the present instance the distance is barely twice as far, but the writer has already commented on the fact that the character is probably an unstable one.

The pupa is light brown with rather short thick abdomen. The dorsal portion of each abdominal segment II-VIII is strongly rugulose as far back as the caudal row of spines, the remaining posterior sections being covered by a very fine network. The transverse rows of spines are greatly reduced and scarcely visible above the general surface rugosity, particularly the caudal row. The segments IX and X are smooth dorsally, without spines. The cremaster is short, much broader than long and bent sharply dorsad, almost at right angles; its terminal edge is provided with four equidistant spines, two median and two lateral with a fine seta midway between each outer pair; there is also a single lateral seta and a small one dorsally near the base. The anal rise is improminent and without setae. The front is smooth.

*Peronea cornana* McD.

Larvae of this species were very plentiful in June in laterally rolled leaves of *Cornus paniculata* and allied dogwoods. Superficially these tubular rolls are very similar to those of *Exartema cornanum* but on opening them the

larva is found to live in a silken tube; further, the frass is not ejected, but accumulates within the rolled leaf. Pupation takes place loosely within the roll, generally in, or close to, the latest fold.

The very active larva has a rather flat pale reddish head with the mouth parts shaded with brown and the antennae moderately long; a patch around the ocelli and a posterolateral streak on the same level, deep black-brown. The setal arrangement is much as in *directana* except that the posterior epicranial setae with their equidistant puncture form almost a straight, somewhat oblique line.

Entire body, including the legs and prothoracic plate, pale green with at times a brownish shade along the anterior margin of the prothoracic plate; pinacula pale, not well defined; anal plate with rather broadly rounded apex, more hemispherical than conical, with the basal edge not distinctly defined; anal fork well developed, five- to six-pronged, spiracles pale, whitish. On the ninth abdominal segment seta *alpha* is situated considerably more forward of the line between *beta* and *rho* than is the case in *directana*, and the *Pi* group is bisetose on both segment VII and segment VIII and occasionally unisetose on IX; this character is given generic value by Fracker. Judging by the present species, Forbes' figure (6) of the setal arrangement of a *Peronea* sp. is inaccurate as far as the ninth abdominal segment is concerned; the *beta* setae are shown as separate instead of on a single pinaculum, *alpha* is too close to *rho* and the *Kappa* group should be on a single pinaculum (as in his Fig. 245) instead of being represented as separate.

The light brown pupa corresponds excellently with the characterization given by Mosher for the genus *Peronea*.

### Eucosminae

#### *Epinotia lindana* Fern.

Dr. Dyar commented briefly on the larva of this species (3, p. 928) giving the food plant as *Cornus*.

In the early spring the writer found the larvae very plentiful, tying the young terminal leaves of *Cornus paniculata* and allied species; as noted by Dr. Dyar the leaves so tied were mostly killed and formed an unsightly bunch among the fresh green foliage. The sluggish grub-like larva was generally found in the centre of the mass in a fold of a dried leaf. Under natural conditions the larvae apparently leave the plant when ready for pupation about the middle of June and spin a rather firm cocoon under rubbish on the ground; pupation does not actually take place for a month or six weeks, the larva remaining unchanged in its cocoon until August. The actual pupal period lasts about two to three weeks, the moths emerging at the end of August and during September.

The larva is dull whitish or dirty greenish with the head, prothoracic plate, pinaculum of the *Kappa* group on the prothorax and the legs, brown. In mature larvae the anterior portion of the prothoracic plate is shaded with paler color; on the head the labrum, patch around the ocelli, frontal sutures

and rear margins of epicranial lobes are deep black-brown. The setal arrangement of the head is shown in Fig. 5 and it should be noted that the posterior epicranial setae have moved forward considerably as compared with those of the true Tortricids (see *Sparganothis directana*, Fig. 1); also that the position of the anterior epicranial puncture (as far as can be ascertained, it being very difficult to locate in this species) is *posterior* to both its setae. On the prothorax setae *gamma*, *eta* and *rho* form more or less of an equilateral triangle and in the *Kappa* group the middle seta is situated ventrad of a line between the other two; on the other thoracic segments and on the first seven abdominal segments the position of the setae is normal, except that on VII the *Pi* group is bisetose (not trisetose as on preceding segments) and remains so on the following two segments. On segment VIII the spiracle has moved up well dorsad of *rho* and on IX *alpha* is associated with *rho* on a single pinaculum, and the three setae of the *Kappa* group in a vertical row on one pinaculum. The spiracles are pale with brown ring; abdominal pinacula large but improminent, anal plate hemispherical, occupying almost the entire dorsum; anal fork weak, consisting of three or four short prongs; hooks of prolegs biordinal, forming a complete circle. By Fracker's key (7) the species would trace to the genus *Eucosma*.

The pupa is light brown. On segments II-VII there are double transverse rows of dorsal spines, the caudal row consisting of much smaller spines than the cephalic one; on segments VIII-X only the cephalic row is present, those on X being larger than on IX and arranged in a semicircle around the slightly raised, blunt, caudal end. The cremaster is lacking, but there is a dorsolateral and a ventrolateral seta on each side of the caudal end and a lateral seta arising from a distinct papilla on the anal rise. There are no furrows or pits on the abdominal segments. The front shows a small conical rise. The pupa does not fit in at all with Mosher's keys (12) to the genus *Epinotia* (based on *saliciana* Clem. and *piceaefoliaria* Kft., only the latter species being now included in the genus) but falls rather to *Enarmonia* (based on *fana* Kft., a species now placed in *Grapholitha*).

*Epinotia myricana* McD.

The larvae of this species were not uncommon on *Myrica*; as in the preceding species they are rather sluggish and grub-like. They mature early in June and in the breeding tins spun a rather firm cocoon under paper or refuse, pupating in from 10 days to two weeks; the pupal stage lasts approximately a month, the moths emerging singly and irregularly during the last three weeks of July.

The larva is dirty white with the entire dorsal area suffused with dull greenish and very finely shagreened, the rather large pinacula remaining pale. The head is pale greenish white with considerable brownish marbling on the epicranial lobes posteriorly and laterally; the labrum, a patch around the ocelli, the frontal sutures and the posterior margin of the lobes deep black-brown. The setal arrangement shows the following differences from that of *lindana*: the posterior adfrontal seta has moved forward in about a line

with the first posterior epicranial one, the two adfrontal setae in consequence being very close together; the anterior epicranial suture has moved forward slightly to the same level as the second anterior seta.

The prothoracic plate is pale with sparse brown sprinkling; the setal arrangement of the prothorax is much as in *lindana* but in the *Kappa* group the middle seta is almost in alignment with the other two and only slightly nearer to the cephalic seta than to the caudal one. On the other thoracic segments the *Pi* group may be bisetose; a larva has been examined which is bisetose on one side and trisetose on the other, so that the lack of a seta has evidently no classificatory value. On abdominal segment VII the *Pi* group may be either bisetose or trisetose; on VIII the spiracle is hardly as far dorsad as in *lindana*; on IX the arrangement is practically identical, the pinacula of the two *beta* setae and of *alpha* and *rho* being normally fused. Anal plate pale, hemispherical as in *lindana*; anal fork much stronger than in *lindana*, composed of seven prongs. Spiracles pale, black ringed, with a faint brownish surrounding shade.

The brown pupa is almost identical structurally with that of the preceding species, except that in some cases there are two further fine setae situated at the caudal end nearer the median line, besides the four already noted; this, however, is by no means a constant feature. The pupa of *E. solandriana* Linn. also agrees in structural details with the present two species, showing distinctly in the one specimen examined (bred from larva on birch at Bobcaygeon, July 6) the six terminal setae.

#### *Anchylopera semiovana* Zell.

In both 1931 and 1932 the moths of this species were extremely abundant during the latter half of June and early July around bushes of *Ceanothus americana* from which they were readily beaten. In spite of repeated searching no trace of possible larvae could be found until early August, 1932, when very small larvae were taken on *Ceanothus*, living either in a slight fold at the edge of a leaf or in a longitudinal fold along one of the ribs. These were brought back to Ottawa and, by forcing, a partial second and rather under-sized generation of the present species was secured in September; under normal conditions it seems likely that, in Canada at least, the larva hibernates nearly full grown.

The larva is light yellow-green with rather prominent pale pinacula on the abdomen, and a well-defined division of each segment into two obliquely semiequal subsegments, the posterior one of which may again be subdivided into dorsal and lateral portions. The head and both prothoracic and anal plates are paler than the dorsum of the abdomen; the former has the mandibles light brown, a deep black-brown area around the ocelli and a small posterolateral blackish spot on a level with the ocelli; the prothoracic plate has a prominent round blackish spot in the posterolateral angle and the anal plate is crossed just posterior to the first row of setae, by a lunate dark brown band. Anal fork well developed, six pronged; legs pale; spiracles pale with black ring.

Notwithstanding the fact that Heinrich (9, p. 243) in his revision derives the genus direct from *Epinotia*, the larva of the present species, by the arrangement of both its head setae and those of the ninth segment, shows far closer relationship to the true Tortricids than to *Epinotia* and in this respect Fracker's reference of the genus to a group with *Tortrix* and *Cenopsis* is confirmed. The setal arrangement on IX is as in the writer's figure of *directana* except that the *alpha* pinaculum has moved somewhat more cephalad; the same similarity exists between the ventral setae on the posterior segments; the anal plate has the posterior margin less evenly rounded than in *Epinotia lindana* and a tendency to be somewhat conical. On the prothorax the middle seta of the *Kappa* group is ventrad of a line between the other two and about twice as far from the posterior one as from the cephalic one; on the head the arrangement is much as in *directana* except that the anterior epicranial puncture is closer to  $A_2$  than to  $A_1$ .

The pale brown pupa corresponds in all respects with Mosher's characterization of the genus *Ancylis*.

### Argyroplocinae (Oleuthreutinae)

#### *Exartema cornanum* Heinr.

The larvae were obtained quite readily in tightly, laterally rolled leaves of dogwood (*Cornus* spp.) in early June; these spiral rolls are quite similar to those of *Peronea cornana* outwardly, but differ when opened out in that the larva does not live in a silken tube and that all frass is ejected from the roll and not left inside it, as is the case with *Peronea*. The larva pupates within the tube, turning down the edge of the leaf to form a compact cocoon; the pupa is readily secured by hunting for the characteristic spiral leaf-rolls in late June, although it is often found that at this time either birds have picked open the roll and obtained the pupa or that *Macrocentrus* cocoons have taken its place.

The larva is pale green with light reddish head and prothoracic plate; on the head a patch around the ocelli and a posterolateral streak on the same level as the ocelli are black-brown; the pinacula are moderate in size and somewhat paler than the ground color; the anal plate is conical. The position of the setae on the head capsule (Fig. 8) seems closer to that occurring in the Tortricids, especially in species of the genus *Cacoecia*, than to *Epinotia*; the frontal sutures, however, are perfectly V-shaped and not concave in the dorsal third which neither agrees with the figure of *Sparganothis* nor with Fracker's key. Nothing specially different in the setal arrangement on the thoracic and first eight abdominal segments can be found; the three setae of the *Kappa* group on the prothorax are in alignment; on all abdominal segments *kappa* and *eta* are almost vertically placed, *eta* being very slightly caudad of *kappa*. On segment IX seta *alpha* is closer to *rho* than to *beta* (see figure) but not on a single pinaculum with it and well anterior to both it and *beta*; *mu* is on the same pinaculum with *kappa* and *eta* and situated very slightly caudad of the other two which are placed vertically. The anal plate is typically Tortricid and the anal fork well developed, with seven prongs.

The dark brown pupa runs to *Exartema* (a) in Mosher's keys; it has a strong frontal prow, which, viewed dorsally, is tongue-shaped and from the side roughly triangular.

It might be noted that larvae of *Exartema punctanum* Wlshn, were taken along with those of *cornanum* but not differentiated from them; the very similar pupa, however, was found to have apparently a decidedly paler brown color on the wing sheaths in *punctanum* (deep brown in *cornanum*) and to emerge, on the whole, a week or two later. Most of the writer's material of this species was collected as pupae from the spirally rolled leaves.

*Exartema permundanum* Clem. (?)

From larvae obtained on *Myrica* in June, along with those of *Cacoecia myricana*, *C. parallela* and *Epinotia myricana*, adults were bred which appear to belong to a dark form of this species. On the primaries the dark areas are either partly or entirely covered with ruddy scaling which seems to be entirely lacking in the typical form (as determined by C. Heinrich based on Clemens' type specimen) in which these areas are olivaceous over a brown base; the secondaries too in both sexes are deeper black than in typical *permundanum*. There are no tangible differences in either the male or female genitalia and until more definite information is available regarding the larva of the typical form and its food plants, as well as of the variety *gaylussacianum* (a Kearfott Mss. name which was validated by Heinrich in his revision, page 155, for the huckleberry feeder), it scarcely appears advisable to name the present form.

The larva is light green with black head and prothoracic shield and some blackish shading on the prothoracic pinacula and on the legs, notably those of the prothorax. The skin, under a moderate magnification appears finely but definitely shagreened. The general structure and setal arrangement is much as in the preceding species; on the head, however, the frontal sutures are more definitely concave in the dorsal third and the species, therefore, fits in better with Fracker's key; in the arrangement of the ocelli the second is somewhat closer to the third than to the first. The anal plate is more bluntly conical, the apex of same being broader than in *cornanum*; the anal fork is well developed with six rather long prongs of a darkish color. The pupa is without the cephalic prow of *cornanum* and very similar to that of the following species. The larvae of this and of the following species are extremely difficult to differentiate from half-grown larvae of *Cacoecia rosaceana* Harr. which occur on the same food plants. The closer association of *alpha* with *rho* on the ninth segment in the *Exartema* species is generally helpful in distinguishing the species, but the writer has noted that in a certain proportion of *rosaceana* larvae, *alpha* is certainly somewhat closer to *rho* than to *beta*, although normally equidistant. An additional point of distinction is that the anal plate in *rosaceana* is much less conical with an almost rounded apical margin. Mature larvae of *rosaceana* are naturally much larger and show a marked tendency for the head and prothoracic plate to become either partly or entirely pale brown.

*Exartema valdanum* McD.

A small series of this species was bred from larvae obtained on *Spiraea salicifolia* in June along with those of *Evora hemidesma* Zell. and *Argyroploce albiciliana*, all of these species tying the terminal leaves of the plant. Clemens in his original description of *permundanum* mentions *Spiraea* as the food plant of the species; it is, however, to judge by the description, quite probable that his series was mixed and that the bred specimen actually belonged to what we now know as *valdanum*; in any case 12 bred specimens of the species described here all show very constantly the white antemedian band of the forewing which characterizes *valdanum*.

The very active larva is pale green with black head and prothoracic plate and some blackish suffusion on the prothoracic pinacula, especially the *Kappa* one, as well as on the legs, which are partly pale brown. It is very similar to the larva of the preceding species but shows the following slight points of difference: the skin is less evidently shagreened; on the ninth abdominal segment the *beta* setae are on separate pinacula (possibly not constant); the anal plate is slightly narrower at the apex and the prongs of the anal fork are shorter; weaker and paler in color.

Pupation occurs early in June and the light brown pupa is also without a prow; the slightly raised portion of the front being roughly dumb-bell shaped. It agrees in other respects with Mosher's characterization of the genus *Exartema* (a).

*Argyroploce albiciliana* Fern.

Apart from Heinrich's record of *Spiraea salicifolia* as the food plant, based on Kearfott's notes\*, the writer knows of no larval records of this species.

The rather sluggish, grub-like larvae were not uncommon tying the terminal shoots of the above-mentioned plant in June and are at once recognizable by their entirely blackish coloration. The head, legs and anal plate are shining black, remainder of body, including the prothoracic plate and abdominal pinacula, deep, dull black-brown very finely shagreened. While there appears to be nothing very different in the setal arrangement on the head from that of *Exartema cornanum*, there are a number of differences in the position of the body setae; in the three aligned setae of the *Kappa* group on the prothorax the middle seta is almost equidistant between the caudal and cephalic setae, being only slightly closer to the latter, instead of at least twice as close; on the first two and the seventh abdominal segments the *Pi* group is bisetose, and unisetose on the eighth and ninth segments; on the eighth abdominal segment the *beta* setae are already somewhat closer together than the *alpha* ones, whilst on the ninth segment they are extremely close to each other and *alpha* and *rho* are situated on a single pinaculum, the area of skin between the two *rho* pinacula being more lined and roughened than

\*See Reference 10.



on the remainder of the segment. The anal plate is conical, rather more bluntly so than in the figure of *Exartema cornanum*; the anal fork is strong, consisting of four stout, blunt, blackish prongs.

The dark brown pupa has a well-developed cephalic prow, narrowly cylindrical with two apicolateral tubercles, the whole with considerable resemblance to the head of a Neuropterous insect; there is a further tubercle at the base of each antennal sheath. The cremaster is greatly reduced, being represented at the best by two small triangular lateral projections with the usual number of rather thin setae between them; there are two similar setae on each side of the anal rise and no spines dorsally on the tenth segment.

*Evora hemidesma* Zell.

The larva of this species is briefly recorded by Kearfott (11) as feeding on *Spiraea tormentosa*. The writer found them about equally common with the preceding species on *Spiraea salicifolia* in June. They are also blackish-brown in coloration of the abdomen but may be readily distinguished by the pale head, prothoracic and anal plates and by the pale yellowish abdominal pinacula.

The head is light yellow-brown, the area around the ocelli and a long posterolateral streak extending forward to the ocellar setae, as well as shading on the mouth parts, dark black-brown; the setal arrangement is much as in *Exartema*, except that the anterior epicranial puncture has moved cephalad to a position almost equidistant between the two setae. The prothoracic plate is colored similarly to the head with two large, irregular, posterolateral black-brown patches. The whole body is black-brown with the exception of the pale pinacula and is finely, but distinctly, shagreened; the legs are blackish. The setal arrangement shows no variation from that found in *Exartema* and differs, therefore, from the preceding species in this respect. The anal plate is pale yellowish with a dark, lunate band starting in the posterolateral corners and curving caudad of the first pair of setae; the anal fork is well developed with six pale-colored prongs.

The black-brown pupa is provided with a frontal prow very similar to that of *Exartema cornanum* as already described; in addition, however, there are tubercles at base of antennal sheaths, slightly smaller and blunter than in the preceding species. There is a short broad cremaster, the apical edge, viewed dorsally, showing two sharp lateral projections; in the medioventral area is a raised ridge, the whole cremaster being very similar to that of *Exartema*, with the usual number of four hooks, two apical and two lateral on each side of the median line. The anal rise is provided with two lateral setae on each side, much smaller than the cremastral hooks and there is a distinct group of spines dorsally on the tenth segment, arranged in several closely approximated rows.

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## STUDIES ON THE INHERITANCE OF COVERED SMUT REACTION, LEMMA COLOR, AWN DEVELOPMENT AND RACHILLA PUBESCENCE IN OATS<sup>1</sup>

By L. P. V. JOHNSON<sup>2</sup>

### Abstract

An oat cross, Black Mesdag × Victory, was studied genetically for covered smut reaction, lemma color, awn development and rachilla pubescence. Only the  $F_2$  was studied for reaction to the covered smut fungus, *Ustilago levis* (K. and S.) Magn. Prior to sowing, the caryopses were dehulled and inoculated with smut spores. Hybrid susceptibility (up to 95%) corresponded with that of Victory, the non-resistant parent. Segregation among  $F_2$  families occurred in the ratio 4 immune : 9 moderately resistant : 3 susceptible. It is concluded that smut resistance is conditioned by two genetic factors: a dominant factor, which when homozygous gives high resistance or immunity, and a less potent supplementary factor.

Both the  $F_1$  and  $F_2$  were studied for the grain characters mentioned. Each of these characters was found to be controlled by two genetic factors.  $F_2$  segregation ratios were as follows:—lemma color, 12 black : 3 gray : 1 white; awn development, 12 strong : 3 intermediate : 1 weak; rachilla pubescence, 12 long : 3 short : 1 glabrous. The  $F_2$  gave good substantiation of the  $F_2$  ratios except in the case of awn development where fairly wide deviations from the expected occurred, due, it is believed, to environmental influences.

No correlations between smut reaction and grain characters were found. Association between rachilla and callus pubescence was observed; but this is not believed to be due, necessarily, to a genetic linkage.

Homozygous strains combining smut immunity with agronomically desirable grain characters were obtained.

### Introduction

A genetic study of covered smut reaction and of certain grain characters in hybrids of Black Mesdag and Victory oats is reported herein. The variety Black Mesdag, while of inferior quality, is very resistant to the covered smut fungus, *Ustilago levis* (K. and S.) Magn.; Victory, on the other hand, though of excellent quality, is very susceptible to this pathogen. The primary

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object of the investigation was to record and analyze genetic data on the reaction to the disease and to obtain, or to ascertain the possibilities of obtaining, true-breeding strains possessing the desirable characters of both parents. Genetic data were also compiled on lemma color, awn development and rachilla pubescence for the purpose of studying the mode of inheritance of the character in each case, and of testing its relation to smut reaction and to the other characters studied.

## Materials and Experimental Methods

### *Parental Material*

The cultures used in these studies were  $F_1$  to  $F_3$  generations (complete) and certain  $F_4$  lines of several crosses between Black Mesdag and Victory varieties of oats. In all crosses Black Mesdag was the pistillate and Victory was the staminate parent. A comparison of these varieties for the characters studied is summarized in Table I.

TABLE I  
COMPARISON OF PARENTAL VARIETIES FOR CHARACTERS STUDIED

Variety	Lemma color	Awn development	Rachilla pubescence	Callus pubescence	Av. percentage infection by covered smut*
Victory	White	Usually weak; often slightly twisted, seldom geniculate	Absent	Occasionally a few short hairs	89.2
Black Mesdag C.I. 1877	Black	Strong; twisted, geniculate	Numerous hairs present	Occasionally a few short hairs	0.0

\*Data obtained from parental lines tested with hybrids. See Table II.

Comparison of Black Mesdag with Victory shows that in lemma color and smut reaction the parents are entirely different; the former is black and immune, while the latter is white and susceptible. Black Mesdag has a far stronger development of awns; it has fairly heavy rachilla pubescence which contrasts with the glabrous condition of the other parent. Both varieties are practically the same with respect to callus pubescence.

### *Inoculum and Methods of Inoculation*

Only the reaction to covered smut of oats, caused by *Ustilago levis* (K. and S.) Magn., was studied in the present test. In 1930 a composite was made up of smutted panicles collected from many different varieties in many parts of the province. Inoculum was prepared by grinding the infected panicles of this collection. In 1931 several field smut tests were made which collectively included about 250 varieties and 450 hybrid lines including 127  $F_3$  lines of the

Black Mesdag  $\times$  Victory cross. Smutted panicles were collected from over 200 varieties and from nearly 300 hybrid lines. The composite spore material obtained from this collection served as inoculum for the retesting, in 1932, of the  $F_3$  lines of the present material.

Reed (26, 28, 30) and Reed and Stanton (35) have reported evidence indicating the existence of several physiologic races of both oat smut fungi. It is believed that the inoculum used in the present investigation includes the majority, if not all, of the physiologic forms of *U. levis* existent in the province.

As the 1931 smut test of  $F_3$  lines, in which hulled seeds (hulls not removed from caryopses) were used, resulted in only fair smut infection, it was decided to repeat the test in 1932 using dehulled seeds (hulls removed from caryopses). Fifty seeds, or as many as available up to that number, of each  $F_2$  plant were dehulled and placed in an envelope. The inoculum was prepared by grinding the smutted panicles and removing the chaffy material by sifting. A liberal quantity of inoculum was added to each envelope which was then shaken vigorously. The smut infection in 1932 was very severe. Previous investigators, particularly Johnston (20) and Stanton *et al.* (38), have found that dehulling of the seed increases smut infection considerably.

#### *General Methods of Growing and Handling the Material*

In all, three complete sowings were made from seeds of  $F_2$  plants: in 1931 duplicate sowings were made, one being a genetic nursery used as a source of material for morphological studies, the other being a smut test; in 1932 the smut test was repeated. In all sowings, plots of both parents were grown with the hybrids at 20-plot intervals. All plots were single rows, 10 ft. in length, with 50 seeds (or as many as available) per row.

Notes on smut infection were taken in the field when plants neared maturity. Counts of total number of plants and of number of plants infected were made in each hybrid and parental row. (Plants were spaced approximately  $2\frac{1}{2}$  in. apart to facilitate counting.) From data thus collected the percentage of smut infection was later calculated for each row.

The plants in the genetic nursery plots were harvested by pulling up each plant by the roots and making a sheaf from each line. The data on awn development were compiled for each plant prior to threshing. Data on all other morphological characters of the seed were obtained by examining after threshing. It should be stated that in threshing the outer glumes of many spikelets were not removed. Seeds thus protected were utilized to advantage in classifying for pubescence, as completely threshed grains often lost many hairs in the process.

A study, paralleling the present investigation in every respect, was made of the cross Black Mesdag  $\times$  Banner; the  $F_3$  population was, however, so reduced by the necessity for retesting that the study does not warrant reporting. The results were, throughout, in good agreement with those herein reported.

### *Environmental Conditions*

Bartholomew and Jones (2) found that the optimum temperatures for growth, spore germination, and sporidial production in *Ustilago avenae*, the loose smut fungus, were 68°, 59° and 59° F., respectively. In soil of 36% moisture, 100% infection was obtained at temperatures between 59° and 73° F. Reed and Faris (33) obtained highest infections by *U. levis* at 77° F. with severe infection at 59° and 68° F. Johnston (20) found soil temperatures of 62° to 66° F. to be the most favorable for infection by a mixture of *U. avenae* and *U. levis*.

In the spring of 1932 records of soil temperatures were obtained by means of a thermograph. Seeding was done on May 6 when soil temperatures ranged from 52° to 65° F. with the average at 59° F. Temperatures for the three days preceding had been practically the same, and there was little change in average soil temperatures up to the time of emergence of the seedlings. It is believed that the temperature conditions of the 1932 smut test were near the optimum for smut infection.

### **Inheritance of Smut Reaction**

Literature on the inheritance of smut reaction in oats is comparatively recent, dating from 1921 when Wakabayashi (40) published data on the reaction of oat hybrids to *Ustilago levis*. Since that time nearly a score of investigations on the inheritance of reaction to one or the other or both of the oat smuts have been reported. Conclusions vary regarding the number and nature of the genetic factors concerned and regarding the genetic relationship of reaction to the two smuts. The literature on the inheritance of reaction to *U. levis* and to *U. avenae* is summarized in tabular form in Appendix A.

### *Experimental Results*

As has already been stated, the  $F_3$  lines were tested for reaction to covered smut in both 1931 and 1932. In the present analyses only the 1932 test will be considered as far greater infections were obtained in that year. The correlation coefficient for smut infection in 1931 and smut infection in 1932 was  $+0.823 \pm 0.021$ .

The necessity for three sowings from the seeds of  $F_2$  plants considerably reduced the number of plants in some  $F_3$  families in the 1932 smut test. Only families with sufficient numbers to give a reliable indication of inherent reaction are included in the following analyses. The standard set for the zero-infection class was as follows: no infection in 1931 (based on 40-50 plants), no infection in 1932 (row of 20 or more plants). The standard for the intermediate infection class was a test of 20 or more plants which resulted in infection ranging from a trace to 39.9%. The minimum standard for inclusion of a family in the susceptible class is represented by a family which showed a comparatively very high infection in 1931, and 13 out of 17 plants infected in 1932. In a great majority of the cases the population of 1932  $F_3$  families ranged from 35 to 50 plants.

The classification of  $F_2$  plants for smut reaction on the basis of percentage smut infection of  $F_3$  families, together with parental reactions, is presented in Table II.

TABLE II

CLASSIFICATION OF  $F_2$  PLANTS ON THE BASIS OF SMUT REACTION OF  $F_3$  FAMILIES AND COMPARISON WITH SMUT REACTION OF PARENTS

Material	Class centres in percentage smut infection											Number of plots
	0	5	15	25	35	45	55	65	75	85	95	
Victory												
Black Mesdag	10									4	3	7
Black Mesdag $\times$ Victory $F_2$	28	23	16	18	1	1	2	1	6	5	1	102
Black Mesdag $\times$ Victory $F_2$ grouped	28	58					16					102
Calculated, 4 : 9 : 3 ratio	25.500	57.375					19.125					102.000

*Black Mesdag*  $\times$  *Victory*,  $\chi^2 = 0.772$ ,  $P = > 0.6065$

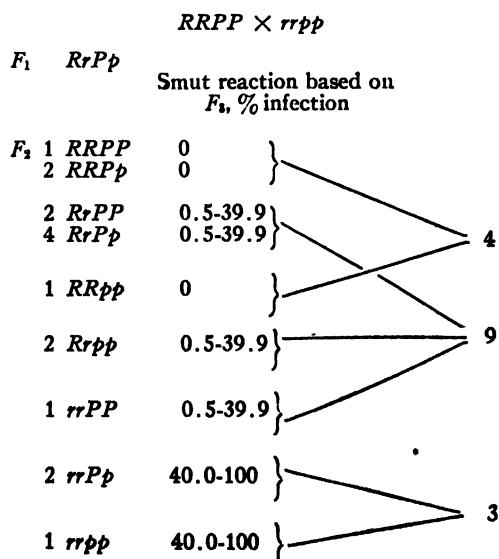
These results could be explained, tentatively, by assuming the following factorial interactions:—Black Mesdag, *RRPP*; Victory, *rrpp*. *R* is a dominant factor for smut resistance which in a homozygous condition gives high resistance or immunity. *P* is a supplementary factor for resistance which is less potent than *R*, and in a homozygous condition gives only partial resistance. The recessive allelomorphs *r* and *p* condition smut susceptibility.

Thus, the factorial interaction, with respect to smut reaction, in the present crosses may be represented by the following diagram.

This hypothesis is in agreement with the genetic explanation suggested by Hayes *et al.* (19) and, it is believed, may be modified so as to agree with the factorial analysis stated by Garber *et al.* (16).

Garber *et al.* (15, 16), in order to explain transgressive segregation for susceptibility in a Gopher  $\times$  Black Mesdag cross, ascribed the following genotypes to the parents:—Black Mesdag, *RRii*; Gopher, *rrII* (see Appendix A).

Except for a narrower range of infection (0 to 67.5%) their data on smut reaction of  $F_3$  families agree with those of the present experiment.





Factorial analyses of both investigations will agree if the following parental genotypes be assumed:—Black Mesdag, *RRPPii*; Victory, *rrppii*; Gopher, *rrPPII*.

The two-factor ratio assumed in each analysis will still hold good. The addition of factors *PP* to the genotype of Gopher accounts for the smaller degree of infection obtained in the cross involving that variety.

As dehulled seeds were used in the present investigation, the fact that Garber *et al.* used hulled seed must be considered when comparing, directly, the infection ranges of the respective cultures. Garber *et al.* reported an average smut infection of 19.5% for the 11 Gopher parental plots grown with the  $F_3$  families. Under the conditions of the present investigation, the average infections obtained for four plots of Gopher were 1.6% when hulled seed was used, and 23.5% when dehulled seed was used. It would appear, therefore, that conditions under which Garber *et al.* grew their material were such that their infection results from hulled seeds are closely comparable to those obtained from dehulled seeds under the condition of the present investigation. The direct comparison of infection ranges made above is therefore, to a considerable extent, justifiable.

It should also be mentioned that Garber *et al.* used as inoculum a mixture of the two oat smuts, while in the present study only covered smut was used.

The  $F_4$  was not grown, except for the growing and testing for smut reaction of certain selections of agronomically desirable types from highly resistant or immune  $F_3$  families. As the object of growing these lines was a purely economic one, no genetic analysis has been made. It might be stated, however, that all families showing immunity to smut in the  $F_3$  were also immune in the  $F_4$ , a behavior to be expected on the basis of the hypothesis.

### Inheritance of Lemma Color

A study of the literature dealing with the inheritance of lemma color in oats reveals an apparent difference in the genetical constitution of certain black-grained varieties (see Appendix B). The recent work of Robb (36) brings out this point with particular clearness.

Reports on the inheritance of lemma color in crosses in which Black Mesdag was the black parent do not agree as to the number of factors involved. Garber and Quisenberry (14) found lemma color to be controlled by one factor in a cross between Black Mesdag and Gopher (white). Lunden (23) and Hayes, Griffie, Stevenson and Lunden (19) obtained similar results in crosses between Black Mesdag and homozygous strains from Minota  $\times$  White Russian and White Russian  $\times$  Victory crosses. Robb (36) observed a two-factor interaction in a cross, Breseler's Prolific (white)  $\times$  Black Mesdag.

This difference in results may be explained in a number of ways: the white-grained parents may have carried, in the cases where only one factor was indicated, a factor inhibiting the expression of the factor for a gray lemma color present in Black Mesdag; the parent designated as Black

Mesdag may not have been the same; the environmental conditions, under which were conducted the experiments that indicated one factor for lemma color, may have been such that the factor for gray lemma color was not expressed.

### Experimental Results

The lemma color of the  $F_1$  grains was similar to that of Black Mesdag. In the  $F_2$ , segregation occurred for black, gray and white in approximately the ratio of 12 : 3 : 1. The  $F_2$  data, together with tests of goodness of fit to the 12 : 3 : 1 ratio, are given in Table III.

The  $P$  value obtained indicates a fairly good fit of the observed to the calculated data. It seems quite safe, therefore, to conclude that the genetic ratio assumed is the correct one.

The  $F_3$  data give good substantiation of the hypothesis based on  $F_2$  results. However, since difficulty was encountered in distinguishing between white and gray colors, in many instances it was impossible to classify the different segregation types. In Table IV certain groupings are made in an attempt to overcome this difficulty.

The observed data are exceedingly close to the expected data based on the  $F_2$  hypothesis, and are in agreement with those reported by Robb (36).

There was some indication of an accumulative effect of the factor for black and also of the factor for gray, but variations in color due to environmental conditions made it impossible to establish the fact.

TABLE III

$F_2$  SEGREGATION FOR LEMMA COLOR IN BLACK MESDAG  
X VICTORY AND THE TEST OF GOODNESS OF FIT  
TO A 12 : 3 : 1 RATIO

Phenotype	Observed <i>o</i>	Calculated <i>c</i>	$\frac{(o-c)^2}{c}$
Black	90	96.0	0.375
Gray	31	24.0	2.042
White	7	8.0	0.125
	128	128.0	$\chi^2 = 2.542$ $P = > 0.2894$

TABLE IV

$F_3$  SEGREGATION FOR LEMMA COLOR IN BLACK MESDAG  
X VICTORY AND TEST OF GOODNESS OF FIT TO  
A CORRECTED 4 : 8 : 3 : 1 RATIO\*

$F_3$ behavior	<i>o</i>	<i>c</i>	$\frac{(o-c)^2}{c}$
Homozygous black	30	30.0	0.0
Heterozygous black	60	60.0	0.0
Homozygous and heterozygous gray	31	31.0	0.0
Homozygous white	7	7.0	0.0
	128	128.0	$\chi^2 = 0.000$ $P = > 0.8013$

\*This is the  $F_3$  ratio of the various types of segregation (with certain groupings) expected from a 12 : 3 : 1  $F_2$  ratio. It is corrected on the basis of actual numbers obtained in the  $F_3$ .

### Inheritance of Awn Development

The inheritance of awn development has been studied in a number of investigations, mainly in connection with interspecific crosses (see Appendix B). The inheritance of the weak awn was studied by Love and Fraser (22) in

*Avena sterilis* var. Burt  $\times$  *A. sativa* var. Sixty Day and Red Texas  $\times$  Burt crosses. A single factor for awns was indicated. Fraser (9) later made a more intensive study of the genetics of the weak awn in crosses between Burt and Sixty Day. The expression of the weak awn was found to be controlled by a single factor. Surface (39), Love and Fraser (22), Fedorova (6) and others have found the strong awn to be strongly linked with the factor for wild type articulation in crosses between *A. fatua* and *A. sativa*. Segregation for awns occurred in the ratio of 1 *sativa* type : 2 intermediate types : 1 *fatua* type.

The mode of inheritance of the strong awn in crosses between varieties of *A. sativa* has not been established. Lunden (23) studied the inheritance of the strong awn in crosses between Black Mesdag and three purified hybrid lines from crosses between Victory or Minota and White Russian. No classification seemed possible because of nearly complete gradations between parental extremes. The data, however, indicated the presence of one main factor for geniculate awns, with other factors probably concerned. Quisenberry (25) found, in a cross between *A. sativa* var. Victor (strong-awned) and *A. sativa orientalis* var. Sparrowbill (weak-awned, nearly awnless), that inheritance of awns was controlled by more than one genetic factor. Hayes, Griffie, Stevenson and Lunden (19) stated, in regard to the inheritance of awns in a cross (Minota  $\times$  White Russian)  $\times$  Black Mesdag, that "without doubt several genetic factors are involved". They also stated that awn development is probably greatly influenced by environmental conditions.

### Experimental Results

When the study of the inheritance of awn development was begun, it was assumed that the character was dependent upon several genetic factors, and that it was greatly influenced by environmental conditions; accordingly, great care was exercised in classifying  $F_2$  and  $F_3$  material. Every plant in both generations was described, with respect to awn development, as follows:— (a) range of awn development (9 classes were used); (b) modal awn class; (c) percentage of awned spikelets (10 classes estimated by inspection).

Genetic analyses were attempted, first, on the basis of the strongest awn

expression (*i.e.*, the upper extreme of the range), and second, on the basis of indices of number of awns (based on percentage of awned spikelets per plant). The former basis proved to be the most satisfactory, and it is believed that the data compiled by that method justify the presentation of a scheme of inheritance or awn development.

The  $F_2$  data, corrected on the basis of  $F_3$  behavior, are given in Table V.

TABLE V

$F_2$  SEGREGATION FOR AWN DEVELOPMENT IN BLACK MESDAG  $\times$  VICTORY AND TEST OF GOODNESS OF FIT TO THE 12 : 3 : 1 RATIO

Phenotype	<i>o</i>	<i>c</i>	$\frac{(o-c)^2}{c}$
Strong (twisted, geniculate)	98	96.0	0.042
Intermediate (twisted, non-geniculate)	21	24.0	0.375
Weak (non-twisted, non-geniculate)	9	8.0	0.125
	128	128.0	$\chi^2 = 0.542$ $P = > 0.6065$

The  $\chi^2$  value indicates an excellent fit of the observed to the calculated data.

The  $F_2$  results may be explained by assuming the following factorial interactions:—Black Mesdag,  $SSu$ ; Victory,  $ssTT$ .  $S$  is a dominant factor for strong (twisted, geniculate) awns,  $T$  is a factor for intermediate (twisted, non-geniculate) awns. The double recessive conditions a few weak (non-twisted, non-geniculate) awns or no awns.

		$SSu \times ssTT$	
$F_1$		$SsTt$	
$F_2$			$F_2$ behavior
12 strong (twisted, geniculate)	(S)	1 $SSTT$	Homozygous $S$
		2 $SsTT$	Segregates $3S : 1T$
		2 $SSTt$	Homozygous $S$
		4 $SsTt$	Segregates $12S : 3T : 1st$
		1 $SStt$	Homozygous $S$
3 intermediate (twisted, non-geniculate)	(T)	2 $Sstt$	Segregates $3S : 1st$
		1 $ssTT$	Homozygous $T$
1 weak (non-twisted, non-geniculate)	(st)	2 $ssTt$	Segregates $3T : 1st$
		1 $ssst$	Homozygous $st$

The existence of awnless and nearly awnless hybrids indicates the possibility of a third genetic factor which operates in the absence of  $S$  and  $T$ , thus changing the ratio from  $12 : 3 : 1$  to  $48 : 12 : 3 : 1$ . Of course, these types may be only variations due to the environment.

The  $F_3$  data do not give a conclusive substantiation of the hypothesis based on the  $F_2$ . It is believed that the influence of environmental factors accounts for the lack of close correspondence between the two generations. Table VI presents the  $F_3$  data classified according to the expected ratio based on the  $F_2$  hypothesis.

A poor fit is indicated by the  $\chi^2$  test in Table VI. It will be noticed that frequencies are low in homozygous classes other than the recessive class. This is to be expected where external factors exert strong influence on genetic material. It seems fair to assume that many genotypically homozygous types, whose expressions are modified by the environment, would, therefore, appear to be segregating.

TABLE VI

$F_3$  SEGREGATION FOR AWN DEVELOPMENT IN BLACK MESDAG  $\times$  VICTORY AND TEST OF GOODNESS OF FIT TO A CORRECTED  $4 : 4 : 2 : 2 : 1 : 2 : 1$  RATIO\*

$F_3$ behavior	$o$	$c$	$\frac{(o-c)^2}{c}$
Homozygous $S$	19	32.67	5.720
$12S : 3T : 1st$	43	32.67	3.266
$3S : 1T$	16	16.33	0.066
$3S : 1st$	20	16.33	0.825
Homozygous $T$	2	7.00	3.571
$3T : 1st$	19	14.00	1.786
Homozygous $st$	9	9.00	0.000
	128	128.00	$\chi^2 = 15.234$ $P = 0.0187$

\*This is the  $F_3$  ratio of the various types of segregation expected from a  $12 : 3 : 1$   $F_2$  ratio. It is corrected on the basis of actual numbers obtained in the  $F_2$ .

### Inheritance of Rachilla Pubescence

The inheritance of rachilla pubescence has not been extensively investigated (see Appendix B). In crosses between *Avena fatua* and *A. sativa*, Surface (39) and Fedorova (6) found rachilla pubescence to be linked with

the factor for *fatua* type articulation, segregation for pubescence occurring in the ratio of 1 *sativa* type : 2 intermediate types : 1 *fatua* type. Lunden (23) reported that pubescence of the rachilla is controlled by a single factor in Black Mesdag crosses. The  $F_1$  generation was intermediate. Hayes, Griffie, Stevenson and Lunden (19) in a study of (White Russian  $\times$  Victory)  $\times$  Black Mesdag and (White Russian  $\times$  Minota)  $\times$  Black Mesdag crosses, observed segregation in the ratio of 3 (few hairs) : 1 (many hairs). Odland (24), working with a cross between Early Gothland (*A. sativa*) and Garton 784 (*A. sativa orientalis*), found segregation for rachilla pubescence in the ratio of 3 smooth : 1 hairy.

### Experimental Results

Two methods of studying the inheritance of rachilla pubescence were used, throughout the present investigation; first, length of hairs and, second, number of hairs was used as the unit of inheritance. A strong, positive correlation exists between these two aspects of rachilla pubescence (see Table X). Both systems of classification were utilized in working out an inheritance scheme, though phenotypic classes are based, primarily, on length of hairs.

Originally, the  $F_2$  data were classified on the basis of presence and absence of hairs. The data fitted the ratio 3 pubescent : 1 glabrous very well. When the  $F_3$  was examined, however, it was found that most of the glabrous types produced hairs, indicating the presence of interclasses within the original recessive group. The  $F_2$  material was then reclassified with great care; a magnifying glass was used to aid in the detection of the slightest indication

TABLE VII

$F_2$  SEGREGATION FOR RACHILLA PUBESCENCE IN BLACK MESDAG  $\times$  VICTORY AND TEST OF GOODNESS OF FIT TO THE 12 : 3 : 1 RATIO

Phenotype	<i>o</i>	<i>c</i>	$\frac{(o-c)^2}{c}$
Long	95	96.0	0.010
Short	25	24.0	0.042
Absent	8	8.0	0.000
	128	128.0	$\chi^2 = 0.052$ $P = > 0.6065$

of pubescence. Classification was based on six classes of pubescence as well as the glabrous class. It was found that there are, apparently, two phenotypic classes of pubescence, long hairs and short hairs; the long hairs were relatively abundant while the short hairs occurred in small numbers—often the inspection of several grains would disclose only a single hair.

Table VII summarizes the

$F_2$  data as corrected on the basis of  $F_3$  behavior.

The fit of the observed to the calculated data is very close.

The  $F_2$  results may be explained by postulating the following hypothesis:—Black Mesdag, *NNFF*; Victory, *nnff*. *N* is a dominant factor which conditions numerous, fairly long hairs on the rachilla. *F* is a supplementary

factor, hypostatic to  $N$ , which gives a few short hairs on the rachilla. The double recessive produces a glabrous condition.

The expression of rachilla hairs in the  $F_3$  was stronger than in the  $F_2$  owing to environmental influences. It was found that the environment affected the length more than it did the

number of hairs; that is to say, while the short hair expression of the  $F_2$  might be "stepped-up" to medium length in the  $F_3$ , the number of hairs associated with short expression remained more or less constant in both generations. This fact was utilized in distinguishing  $F_3$  phenotypes in terms of the  $F_2$  expressions. The data thus compiled afford an excellent substantiation of the  $F_2$  hypothesis.

The  $P$  value calculated in Table VIII indicates an excellent fit between observed and calculated ratios.

The  $F_3$  breeding behavior of plants in the glabrous class of the reclassified  $F_2$  indicated the presence of still another supplementary factor or other modifying condition. Among the eight plants classified as glabrous, two bred true while six segregated as follows for hairlessness and one or more very short hairs per plant, respectively: 34 : 4, 30 : 4, 31 : 6, 25 : 10, 16 : 13, 24 : 19. In the first three cases the hairs were distinctly longer and more numerous than in the last three instances, where the hairs were very minute.

$NNFF \times nnff$

$F_1$	$NnFf$	
$F_2$		$F_2$ behavior
	1 $NNFF$	Homozygous $N$
	2 $NnFF$	Segregates 3 $N$ : 1 $F$
	2 $NNff$	Homozygous $N$
12 numerous, long (N)	4 $NnFf$	Segregates 12 $N$ : 3 $F$ : 1 $nf$
	1 $NNff$	Homozygous $N$
	2 $Nnff$	Segregates 3 $N$ : 1 $nf$
3 few, short (F)	1 $nnFF$	Homozygous $F$
	2 $nnFf$	Segregates 3 $F$ : 1 $nf$
1 absent (nf)	1 $nnff$	Homozygous $nf$

TABLE VIII

$F_3$  SEGREGATION FOR RACHILLA PUBESCENCE IN BLACK MESDAG  $\times$  VICTORY AND TEST OF GOODNESS OF FIT TO A CORRECTED 4 : 4 : 2 : 2 : 1 : 2 : 1 RATIO\*

$F_3$ behavior	$o$	$c$	$\frac{(o-c)^2}{c}$
Homozygous $N$	34	31.67	0.1714
12 $N$ : 3 $F$ : 1 $nf$	34	31.67	0.1714
3 $N$ : 1 $F$	16	15.83	0.0018
3 $N$ : 1 $nf$	11	15.83	1.4737
Homozygous $F$	6	8.33	0.6517
3 $F$ : 1 $nf$	19	16.67	0.3257
Homozygous $nf$	8	8.00	0.0000
	128	127.99	$\chi^2 = 2.7957$ $P = 0.8315$

\*This is the  $F_3$  ratio of the various types of segregation expected from a 12 : 3 : 1  $F_2$  ratio. It is corrected on the basis of actual numbers obtained in the  $F_3$ .

## Studies on Correlated Inheritance

### Correlation between Smut Reaction and Other Characters

The detection of linkage relations between disease reaction and morphological characters is of economic importance because it gives an indication of the possibilities of combining desirable expressions of the latter with disease resistance by hybridization. When the mode of inheritance of the disease

reaction in question is difficult to study by direct methods, correlations with characters of known inheritance provide a basis for an indirect method of attacking the problem.

Review of the literature on this topic was given in connection with the historical review of studies on the inheritance of smut reaction (see Appendix A).

The formulas used in the present correlation studies are those given by Hayes and Garber (18, pp. 43-48).

Correlations between percentage smut infection and the grain characters, lemma color, awn development, rachilla pubescence and callus pubescence were calculated by the correlation ratio method. When this method indicated significant association the correlation coefficient was also calculated. The population consisted of 102  $F_3$  families except when callus pubescence

TABLE IX

CORRELATION BETWEEN SMUT INFECTION  $F_3$  AND CERTAIN MORPHOLOGICAL CHARACTERS

Morphological characters	$\eta_{xy}$	$r_{xy}$
Lemma color $F_2$	$-0.044 \pm 0.067$	—
Strength of awns $F_3$	$+0.323 \pm 0.060$	$+0.010 \pm 0.067$
Number of awns $F_3$	$-0.312 \pm 0.060$	$-0.020 \pm 0.067$
Length of rachilla hairs $F_3$	$+0.123 \pm 0.066$	—
Number of rachilla hairs $F_3$	$-0.323 \pm 0.060$	$-0.097 \pm 0.066$
Length of callus hairs $F_3$	$-0.129 \pm 0.085$	—
Number of callus hairs $F_3$	$-0.202 \pm 0.084$	—

was one of the variables, in which case it was 75  $F_3$  families. A summary of the constants calculated is presented in Table IX.

The correlations obtained by the correlation ratio method between percentage smut infection and the characters strength of awns, number of awns and number of rachilla hairs are significant in the light of their respective probable errors. However, as some obvious defects, which are not taken into account by the probable error, have been found in the method of calculation, it is, nevertheless, concluded that no significant relationships exist between the variables in question.

To explain this conclusion it will be necessary to examine the reliability of the correlation ratio method as a measure of correlation in the present study. The correlation ratio is based on the assumption that if no correlation exists in a given surface, the mean of each row (or column)  $\bar{X}_y$  will be equal to the mean of the entire distribution  $\bar{X}$ . Therefore,  $\bar{X}_y - \bar{X} = 0$ . Completing the computation,  $\sqrt{\sum [f_y(\bar{X}_y - \bar{X})^2] / N} = 0$ . This value is the numerator of the fraction which equals  $\eta_{xy}$ . Therefore,  $\eta_{xy} = 0$ . An increase in the association between the variables will produce a proportional increase in  $\eta_{xy}$  by increasing the difference  $\bar{X}_y - \bar{X}$ . It will be seen that if the frequency of each row is not large enough to give a reliable row mean, the resulting value of  $\eta$  cannot be reliable. This fact is illustrated by the following diagram:

Y ↓ INDICES OF NUMBER OF AWNS $F_2$	X → PERCENTAGE SMUT INFECTION $F_1$											$f_y$	$f_y(\bar{X}_y - \bar{X})^2$
	0	5	15	25	35	45	55	65	75	85	95		
1										1		1	44.22
2	1	3		1	1			1				7	0.07
3	3	1	1									5	15.30
4	1		2	2			1					6	0.60
5	3	3		4					1			11	0.77
6	4	1	4	1		1			2			13	0.52
7	4	6	3	3						1	1	18	0.36
8	4	6	3	2			1		3	1		20	5.00
9	8	3	1	2						2		16	4.64
10			2	3								5	0.30
	28	23	16	18	1	1	2	1	6	5	1	102	71.78

In the above surface the single individual in the top row contributes over two-thirds of the value to the summation  $\Sigma[f_y(\bar{X}_y - \bar{X})^2]$  and its removal would reduce the value of  $\eta$  to insignificance. In the present study it was found that it was those rows which pass through the borders of the scatter and which have very small frequencies that contributed most to the summation referred to above. This error is not taken into consideration by the probable error as calculated, which for given values of  $\eta$  and  $N$  (population) remains the same regardless of the shape of the scatter. The reliability of the probable error as applied to the correlation ratio method is discussed by Fisher (7, p. 224) who states in part, "attempts have been made to test the significance of the correlation ratio by calculating for it a standard error, but such attempts overlook the fact that, even with indefinitely large samples, the distribution of  $\eta$  for zero correlation does not tend to normality unless the number of arrays also is increased without limit".

The correlation ratio should be applied to non-linear distributions in which, irrespective of the total population, each row or column has a frequency large enough to give a reliable mean. The number of rows should be few in order that each may have, if possible, a large frequency. The total population, of course, should be as large as possible.

It is concluded, in spite of the values of  $\eta$  obtained and for reasons given, that no genetic linkages exist between percentage smut infection and the grain characters, lemma color, awn development, rachilla pubescence and callus pubescence.

#### *Correlations Between Various Grain Characters*

Studies on the correlation existing between grain characters are of economic interest, for they indicate the possibilities of combining desirable expressions of these characters through hybridization. Such studies also contribute to genetical knowledge by disclosing the presence or absence of genetic linkages.

The formula for the coefficient of contingency used in the present study is that given by Hayes and Garber (18, pp. 49-50).



Using the coefficient of contingency method, a significant correlation was shown to exist between number of rachilla hairs and number of callus hairs, and doubtful negative correlations are indicated between number of rachilla hairs and strength of awns and between number of callus hairs and strength of awns (see Table X).

There cannot be much doubt regarding the existence of correlation between rachilla and callus pubescence. However, the presence of such correlations is not, necessarily, definite indication of genetic linkages. It is probable that

TABLE X  
CORRELATION BETWEEN THE VARIOUS GRAIN  
CHARACTERS

Variables	$C_1$
Number of rachilla hairs $F_2$ and length of rachilla hairs $F_3$	$+0.775 \pm 0.048$
Number of rachilla hairs $F_2$ and number of callus hairs $F_4$	$+0.499 \pm 0.117$
Number of rachilla hairs $F_2$ and strength of awns $F_5$	$-0.341 \pm 0.105$
Number of callus hairs $F_4$ and strength of awns $F_5$	$-0.372 \pm 0.128$

there are segregations for physiological characters in the present material. A set of physiological factors which promotes a strong expression of, say, rachilla hairs might be expected to produce a like expression of a similar character, such as callus pubescence, regardless of genetic relationships. Environmental factors, such as soil conditions, may vary in such a way as to

produce physiological differences among the plants, which in turn might result in a correlation between certain characters in a given plant.

When working with closely related characters such as awn development, rachilla pubescence, and callus pubescence in a hybrid population, multiple correlations should be calculated before drawing conclusions regarding the existence of genetic linkages.

### Economic Significance

The control of oat smuts is of great economic importance because of the wide distribution and destructiveness of these diseases. Canadian farmers suffer a yearly loss of nearly seven million dollars from oat smut (17). In Alberta, covered smut, caused by *Ustilago levis* (K. and S.) Magn., is very common, while loose smut caused by *Ustilago avenae* (Pers.) Jens. is relatively rare (4, 5). In 1930, covered smut damage in Alberta fields ranged from 0 to 35%; absence of the disease was apparently due to proper seed treatment (5). In 1931 nearly one-half of the fields examined were infected to a greater or lesser degree by covered smut (4).

Chemical seed treatments are usually quite efficient in controlling oat smuts; but, as such methods are troublesome and add to the cost of production, it would appear that a more desirable means of preventing smut infection would be the breeding and use of smut-resistant oat varieties.

The practical objective of the investigation—the production of constant hybrid strains possessing the desirable agronomic characters of the one parent and the smut resistance of the other—has been realized. One  $F_2$  family

was, apparently, homozygous for white, plump grains, weak awns, strong straw, midseason maturity and smut immunity. Forty-eight  $F_4$  lines from this family were grown and all proved to be constant for the characters mentioned. These lines are probably equal to Victory in quality and equal to Black Mesdag in smut resistance. Each line has been bulked and will be increased separately and tested for yield. Several hundred plant selections of weak-awned, white-grained segregates were made from other smut-immune  $F_3$  families. As the characters white grain and weak awn are recessive and the character smut immunity is homozygous dominant, the selections bred true for these characters in the  $F_4$ . Many promising lines have been observed which will be increased for yield tests.

### Summary

An oat cross, Black Mesdag  $\times$  Victory, was studied genetically for covered smut reaction, lemma color, awn development and rachilla pubescence. Smut reaction was studied in the  $F_3$  only; the other characters were studied in both  $F_2$  and  $F_3$ . Black Mesdag has black grains, strong awns, pubescent rachillas and is very resistant to smut infection. Victory has white grains, weak awns, glabrous rachillas, and is very susceptible to smut infection. In the smut test the caryopses were dehulled and inoculated with spores of *Ustilago levis* prior to sowing. Results of the various phases of the investigation are as follows:—

1. Segregation for smut reaction among  $F_3$  families occurred in the ratio 4 immune : 9 moderately resistant : 3 susceptible. Hybrid susceptibility was as high as 95% and corresponded with the susceptibility of the non-resistant parent. Immune  $F_3$  lines bred true for immunity in the  $F_4$ . It is concluded that a two-factor difference for smut reaction exists between the parents. Black Mesdag possesses a dominant factor, which in a homozygous condition gives high resistance or immunity, and a less potent supplementary factor for resistance. Victory possesses the recessive allelomorphs of these factors.

2. Segregation in the  $F_2$  for lemma color occurred in the two-factor ratio 12 black : 3 gray : 1 white. The  $F_3$  data gave a good substantiation of this ratio.

3. Segregation in the  $F_2$  for awn development occurred in the two-factor ratio 12 strong (twisted, geniculate) : 3 intermediate (twisted, non-geniculate) : 1 weak (non-twisted, non-geniculate). The  $F_3$  data did not show a close correspondence to the expected data owing to environmental influences.

4. Segregation in the  $F_2$  for rachilla pubescence occurred in the two-factor ratio 12 numerous, long hairs : 3 few, short hairs : 1 glabrous. The  $F_3$  data gave an excellent substantiation of the  $F_2$  ratio. Some evidence of an additional factor for pubescence was observed.

5. No significant correlation was found between smut reaction and the various grain characters studied.

6. A moderate degree of positive association was found to exist between rachilla pubescence and callus pubescence. As segregation for physiological factors might account for this association, no definite conclusions are drawn regarding genetic linkages.

7. Homozygous strains, possessing white grains, weak awns and other agronomically desirable characters in combination with smut immunity, were selected for further testing.

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## APPENDIX A

TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF THE REACTION OF OAT HYBRIDS TO *Ustilago levis* AND TO *U. avenae*

Investigators	Materials*	<i>U. levis</i>	<i>U. avenae</i>	Remarks
Wakabayashi, 1921 (40)	Red Rustproof (R) × Black Tartarian (S)	Immunity dominant and due, apparently, to three independent factors		
Barney, 1924 (1)	Fulghum (R) × Black Mesdag (R) Swedish Select (S) × Burt (R) Turkish Rustproof (S) × Gold Rain (M)		Resistance controlled by three independent factors. Resistance controlled by two independent factors. Resistance controlled by one independent factor.	
Reed and Stanton, 1925 (34)	Fulghum (R) × Swedish Select (S)	25 $F_2$ families as resistant as Fulghum, 59 moderately to highly susceptible than Swedish Select	25 $F_2$ families as resistant as Fulghum, 59 moderately to highly susceptible than Swedish Select	Evidence that resistance to both smuts was dependent upon the same genetic factors. No correlation observed between smut reaction and morphological characters
Reed, 1925 (27) "	<i>Avena nuda</i> var. <i>inermis</i> (S) × Black Mesdag (R)		Resistance dominant. Single factor difference between parents	No correlation observed between smut reaction and morphological characters
Gaines, 1925 (11, 12)	Red Rustproof (R) × Black Tartarian (M) Red Rustproof (R) × Abundance (M) Red Rustproof (R) × Large Hulless (S) Red Rustproof (R) × Chinese Hulless (S)	Red Rustproof carried three dominant factors for immunity, any one of which prevented infection One of the factors carried by Red Rustproof did not possess complete dominance in hulless segregates		

APPENDIX A—Continued  
TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF THE REACTION OF OAT HYBRIDS TO *Ustilago levis* AND TO *U. avenae*

Investigators	Materials*	<i>U. levis</i>	<i>U. avenae</i>	Remarks
Hayes <i>et al.</i> , 1928 (19)	(White Russian X Minota) (S) X Black Mesdag (R)	Evidence of two pairs of factors for immunity and resistance, respectively, located in Black Mesdag. Factor for immunity epistatic to factor for resistance†	A mixture of <i>U. levis</i> and <i>U. avenae</i> spores used as inoculum. No correlation observed between smut reaction and awn development, rachilla pubescence or lemma color	
Reed, 1928 (29)	Several crosses involving varieties differing in their behavior to one or both smuts. (Black Mesdag used as resistant parent) Crosses between susceptible varieties	Resistance dominant. Single factor difference  All $F_2$ descendants as susceptible as parents	Resistance dominant. Single factor difference  All $F_2$ descendants as susceptible as parents	A great majority of $F_2$ plants and their progenies behaved similarly to both smuts. Recombination of smut resistance with various desirable characters accomplished
Garber <i>et al.</i> , 1928-29 (15, 16)	Gopher (M) X Black Mesdag (R) and the reciprocal	Smut reaction controlled by a single main factor for high resistance or immunity carried by Black Mesdag, and by at least one supplementary factor of less potency carried by Gopher	A mixture of <i>U. levis</i> and <i>U. avenae</i> spores used as inoculum, the latter form predominating. Some evidence of correlation between smut reaction and lemma color	A mixture of <i>U. levis</i> and <i>U. avenae</i> spores used as inoculum, the latter form predominating. Some evidence of correlation between smut reaction and lemma color
Rosenstiel, 1929 (37)	Crosses between Black Mesdag (R) and several susceptible varieties	Monogenic basis of inheritance with resistance the dominant condition		The recombination of smut resistance with other economically desirable characters was accomplished
Gaines and Smith, 1929 (13)	Markton (R) crossed with a number of varieties	Concluded that Markton carried two independent factors for resistance		

†Statements referring to investigations in which mixed cultures were used are placed midway between the *U. levis* and *U. avenae* columns.

## APPENDIX A—Concluded

TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF THE REACTION OF OAT HYBRIDS TO *Ustilago levis* AND TO *U. avenae*

Investigators	Materials*	<i>U. levis</i>	<i>U. avenae</i>	Remarks
Reed, 1931 (31)	Crosses between Early Gothland (resistant to <i>U. levis</i> , susceptible to <i>U. avenae</i> ) and Monarch (resistant to <i>U. avenae</i> , susceptible to <i>U. levis</i> )	10.4% of inoculated $F_2$ plants were infected	18% of inoculated $F_2$ plants were infected	Factors for resistance to loose and to covered smut inherited independently (compare conclusions in 29, 34 above). A few $F_2$ families were resistant to both smuts. No correlation observed between smut reaction and lemma color
Welsh, 1931 (41)	Victory (S) × (Minotaur White Russian × Black Mesdag) (R)	At least two factors for smut reaction. Resistance dominant	At least two factors for smut reaction. Resistance dominant	The same $F_2$ lines were tested with each smut form separately. Moderately high correlation existed between loose and covered smut infection of $F_2$ lines
Coffman <i>et al.</i> , 1931 (3)	Markton (R) × Early Champion (S) Markton (R) × Ligowa (S) Markton (R) × Swedish Select (S)	Approximately three-fourths of progeny lines from $F_2$ were smutted and one-fourth smut-free, indicating a single factor for smut reaction		No significant correlation observed between smut reaction and the characters panicle length, kernel length, kernel width, presence of awns, prominence of nerves in lemmas and lemma color
"	Markton (R) × Scottish Chief (M) Ilogren (S) × Markton (R) Aurora (M) × Markton (R)	Approximately equal numbers of smutted and smut-free progeny lines from $F_2$		
Reed, 1932 (32)	Crosses between Early Gothland (resistant to <i>U. levis</i> , susceptible to <i>U. avenae</i> ) and Victor (susceptible to both fungi)	18 $F_2$ families were entirely resistant, 27 segregating and 7 susceptible	$F_2$ and $F_3$ were almost completely susceptible	

\*Descriptions of smut reaction of materials are abbreviated as follows:—R, resistant; S, susceptible; M, moderately susceptible.

## APPENDIX B

TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF CERTAIN GRAIN CHARACTERS OF OATS

Investigators	Materials	Lemma color	Awn development	Rachilla pubescence
Nilsson-Ehle, 1909-14. Quoted by Surface (39)		48 black : 12 gray : 3 yellow : 1 white	Much affected by environment. Wide variation in pure lines. Negative correlation with yellow lemma	
Zade, 1912. Quoted by Love and Craig (21)	<i>Avena fatua</i> × <i>A. sativa</i>		1 <i>sativa</i> : 2 intermediate : 1 <i>fatua</i> *	
Surface, 1916 (39)	<i>A. fatua</i> × <i>A. sativa</i> var. Kherson	12 black : 3 gray : 1 yellow	1 <i>sativa</i> : 2 intermediate : 1 <i>fatua</i> . Linked with articulation	1 <i>sativa</i> : 2 intermediate : 1 <i>fatua</i> . Linked with articulation
Gaines, 1917 (10)	<i>F</i> <sub>2</sub> of 10 crosses between 6 white and 3 black varieties	3 black : 1 white		
Zinn and Surface, 1917 (42)	<i>F</i> <sub>1</sub> and <i>F</i> <sub>2</sub> of a cross <i>A. sativa</i> var. Victor × <i>A. nuda</i> var. <i>inermis</i>	3 black : 1 white	3 medium strong to strong : 1 weak	
Love and Fraser, 1917 (22)	<i>A. fatua</i> × <i>A. sativa</i> var. Sixty Day and other crosses		1 awnless : 2 partially awned : 1 fully awned	
Love and Craig, 1918 (21)	<i>A. fatua</i> × <i>A. sativa</i> var. Sixty Day	12 black : 3 gray : 1 white	Gene for yellow lemma color inhibits awns in some varieties	
Fraser, 1919 (9)	<i>A. sterilis</i> var. Burt × <i>A. sativa</i> var. Sixty Day	48 red : 15 yellow : 1 white	1 awnless : 2 partially awned : 1 fully awned, or 3 partially awned : 1 fully awned	
Lunden, 1925 (23)	<i>F</i> <sub>1</sub> and <i>F</i> <sub>2</sub> of crosses between Black Meadag and purified white hybrid lines	3 black : 1 white	One main factor producing geniculate awns. Probably supplementary factors for very strong awns	Controlled by single factor; <i>F</i> <sub>1</sub> intermediate
Quisenberry, 1926 (25)	<i>F</i> <sub>2</sub> and <i>F</i> <sub>3</sub> of cross between <i>A. sativa</i> and <i>A. sativa orientalis</i>	3 black : 1 white	More than one genetic factor indicated	
Hayes <i>et al</i> , 1928 (19)	Crosses between Black Meadag and purified white hybrid lines	3 black : 1 white	Several factors involved. Much influence by environment	3 few hairs : 1 many hairs

\*The term *fatua* refers, with respect to awn development, to a condition similar to that found in *A. fatua*, namely, strong geniculate awns on all grains of the spikelet. The term *sativa* refers to a condition of awn development similar to that of the variety of *A. sativa* used in the cross in question.



## APPENDIX B (continued)

TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF CERTAIN GRAIN CHARACTERS OF OATS

Investigators	Materials	Lemma color	Awn development	Rachilla pubescence
Garber and Quisenberry, 1928 (14)	Gopher $\times$ Black Meadag	3 black : 1 white		
Odland, 1928 (24)	<i>A. sativa</i> $\times$ <i>A. sativa orientalis</i>	3 black : 1 white		3 glabrous : 1 pubescent
Tschermak, 1929, Quoted by Florell (8)	<i>A. sativa</i> $\times$ <i>A. fatua</i>		Complete linkage "wild" articulation and "wild" awn development	
Fedorova, 1930 (6)	<i>A. sativa</i> $\times$ <i>A. fatua</i>	9 black : 3 brown : 3 gray : 1 yellow	1 <i>sativa</i> : 2 intermediate : 1 <i>fatua</i> . Linked with articulation	1 <i>sativa</i> : 2 intermediate : 1 <i>fatua</i> . Linked with articulation
Florell, 1931 (8)	<i>A. fatua</i> $\times$ <i>A. sterilis ludoviciana</i>	3 brown ( <i>fatua</i> ) : 1 gray-white ( <i>ludoviciana</i> )		
	<i>A. fatua</i> $\times$ <i>A. byzantina</i> var. Fulghum		Almost complete linkage between <i>fatua</i> awning and <i>fatua</i> articulation. 1-factor	
	<i>A. sterilis ludoviciana</i> $\times$ <i>A. sativa</i> var. North Finnish		Complete linkage between <i>sterilis</i> awning and <i>sterilis</i> articulation. 1-factor	
	<i>A. sterilis ludoviciana</i> $\times$ <i>A. sativa</i> var. Probsteier		Complete linkage between <i>sterilis</i> awning and <i>sterilis</i> articulation. 1-factor	
	<i>A. sterilis macrocarpa</i> $\times$ <i>A. sativa</i> var. Richland		Complete linkage between <i>sterilis</i> awning and <i>sterilis</i> articulation. 1-factor	
Reed, 1931 (31)	$F_2$ of crosses between Early Gothland and Monarch	3 dark : 1 light		
Welsh, 1931 (41)	Helgira strain $\times$ Banner	1 white : 2 seg. : 1 yellow		
	Richland $\times$ purified white hybrid strain	1 white : 2 white-yellow : 1 yellow		
	Joanette strain $\times$ purified white hybrid strain	12 black : 3 gray : 1 white		
	Helgira strain $\times$ Monarch strain	12 black : 3 white : 1 yellow		
Robb, 1932 (36)	$F_2$ of 6 crosses between 4 black and 3 white varieties	3 black : 1 white		

## APPENDIX B—(concluded)

## TABULAR SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF CERTAIN GRAIN CHARACTERS OF OATS

Investigators	Materials	Lemma color	Awn development	Rachilla pubescence
Robb, 1932 (36)	$F_2$ of crosses between 2 black (incl. Black Mesdag) and 2 white varieties	12 black : 3 gray : 1 white		
	$F_2$ of 16 crosses between 1 black (Orion) and 9 white varieties	60 black : 3 gray : 1 white		

# THE EFFECT OF NITROGEN NUTRITION ON THE PROTEIN AND NON-PROTEIN NITROGEN OF WHEAT<sup>1</sup>

BY A. G. McCALLA<sup>2</sup>

## Abstract

Wheat plants were grown in water cultures varied only with respect to nitrogen. The nitrogen as nitrate was supplied to half the plants continuously to maturity, and to the others only until the time of heading. Though uptake and reduction of nitrate continued in the former for some time after heading, organic nitrogen produced in vegetative parts of the plant after heading was not synthesized to protein but accumulated in the form of non-protein compounds. Regardless of the extent of the nitrogen reserves in vegetative parts, translocation to the kernels during filling took place in about the same proportion. In plants with limited nitrogen supply, translocation to kernels consisted largely of decomposed proteins, and the kernels contained less gluten nitrogen than those of the plants with unlimited nitrogen supply which drew upon both protein and non-protein reserves. The nitrogen fractions of the gluten proteins were unaffected by the nitrogen nutrition of the plants. The total amount of non-gluten nitrogen was apparently also unaffected by the nutrition. Amide nitrogen was the most labile of the nitrogen fractions used.

## Introduction

Although it has been known for many years that the nitrogen nutrition of cereals affects the total nitrogen content of the grain produced, the effect on the distribution of various nitrogen compounds has received little attention. It has become increasingly recognized that total nitrogen determinations are entirely inadequate from a physiological point of view, and a large amount of work on various phases of nitrogenous metabolism has been carried out. To investigate the effect of nitrogen nutrition thoroughly, it is necessary to study the chemical development in the vegetative parts of the plants in relation to the chemical development of the kernels. There have been numerous investigations of the progressive development of kernels, but few in which development of vegetative parts has been related to development of kernels, and none at all where these features of metabolism have been related to nutrition. In the following discussion, no attempt is made to refer to all the studies which have been carried out, but a general survey of the problem is given.

The progressive chemical development of wheat kernels was followed by Woodman and Engledow (39) who determined among other things, protein, non-protein, amino acid and ammonia nitrogen. They found that the protein nitrogen increased, and non-protein decreased, as the kernels ripened, until

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they had reached a dry matter content of about 60%. Amino acid nitrogen decreased from 16.1% to less than 1%, and ammonia nitrogen from almost 20% to about 5%, of the total nitrogen.

A somewhat similar study was made by McCalla and Newton (18), who found that during development of the kernels, non-protein nitrogen decreased from 38 to 8% of the total nitrogen. Amino nitrogen was not determined, but the ammonia values did not agree with those obtained by Woodman and Engledow. The highest value obtained was 1.34% of the total nitrogen, and after the kernels had reached a dry matter content of 60%, this fraction was undemonstrable. It is believed that the small values are more nearly correct as few workers have found such large amounts of ammonia as those reported by Woodman and Engledow except in very acid plants such as begonia (30) and rhubarb (31). In these plants, the course of nitrogen metabolism is very different from that in cereals. Small amounts of ammonia have been found in many plants (19, 26, 27, 35), while some workers have failed to demonstrate its presence at all (7).

Knowles and Watkin (16, 17) followed the progressive development of wheat plants and studied total (16) and protein and non-protein (17) nitrogen in both vegetative parts and kernels. Unfortunately these workers dried all their material before analyzing it so the results obtained cannot be accepted as representing conditions in the green plant. They found a continual increase, with age, in the proportion of total nitrogen which existed in the form of proteins in the vegetative parts. Mothes (20) has shown that as green plants age the proportion of protein nitrogen decreases, and (21) that when nitrogen supply is limited, the new growth continues at the expense of older tissues. As the proportion of older growth increases, the percentage total protein will decrease. In view of these results it must be concluded that the increases obtained by Knowles and Watkin were the result of drying and not of normal development of the plants. McCalla and Newton (18) on the other hand have shown that drying kernels before analysis does increase the proportion of protein nitrogen as compared with that of kernels analyzed when fresh.

One of the most significant things demonstrated by Knowles and Watkin, and one which would not be influenced by the methods they used was that at maturity a relatively enormous amount, 71.6%, of the total nitrogen in the whole plant had been translocated into the kernels.

Davidson and LeClerc (6) reported the effects of nitrate fertilizers on the yield and protein content of wheat. They found that nitrates applied during the early stages of growth increased the yield but scarcely affected the protein content of the grain. Nitrates applied at the time of heading did not affect yield, but increased the protein content of grain and straw. Protein content in this case is more accurately described as total nitrogen content because the protein values given are simply total nitrogen values multiplied by a conversion factor.

Gericke (8, 9) also noted that wheat fertilized at the time of heading contained more total nitrogen than did wheat fertilized in the earlier stages of

growth. He found (8) that some of these high nitrogen samples yielded flours of poorer baking quality than those obtained from wheat of the same variety containing less nitrogen. He suggested that the differences in quality might be correlated with differences in the relative proportions of protein and non-protein nitrogen or of glutenin and gliadin in the wheat kernel. He thinks it not improbable that the samples which produced bread of poor quality could have contained relatively large amounts of non-protein, and therefore relatively small amounts of protein, nitrogen, and that the amount of gliadin and glutenin might be much lower in the grain that was high in total nitrogen as a result of late applications of nitrogen fertilizers than in grain low in total nitrogen. Whatever these relations may be, Gericke thinks that the poorness of some of the grain high in total nitrogen was due, at least in part, to overfeeding of the plants, but that the ill effects could be overcome by prolonged growth of these overfed plants. He bases the last statement on results obtained from varieties in which the maturation of high nitrogen samples was delayed by prolonged growth, and on the results obtained by other workers (39) showing that considerable time is required for the transformation of non-protein nitrogen compounds into proteins.

Blish (1) studied gluten and non-gluten proteins of wheat flour and found a wide variation in their proportions in flours of varying protein content and baking characteristics. Those flours which were high in total nitrogen content were also high in the proportion of the total nitrogen which existed in the form of gluten proteins. Although this is essentially the opposite relation to that suggested by Gericke, it must be remembered that Blish was discussing flours in general while Gericke was discussing groups of samples of the same variety grown under varying nutritional conditions.

Grewe and Bailey (12) studied the value  $\frac{\text{glutenin}}{\text{gliadin} + \text{glutenin}}$  in 17 flours of widely varying types. They found a variation of from 0.41 to 0.49 for the value, or in other words, glutenin varied from 41% to 49% of the gluten proteins. These workers state that the differences are so small that they justify the conclusion that there is no substantial variation in the proportions of gliadin and glutenin in the flours studied.

Recently Gericke (9) has indicated that he now considers that the quantitative distribution of the various nitrogen-containing compounds has little or nothing to do with determining the quality of wheat flour. He now believes that the quantity and nature of the inorganic salts absorbed by the plant during the later stages of growth play an important role in determining the physical properties of the gluten and starch in the wheat kernels. These properties in turn determine flour quality.

The writer believed that by varying the amount of nitrogen supplied to wheat and following the development through the early stages of growth as well as during the formation and ripening of the kernels, some more definite information relating to the nitrogen compounds might be obtained. Two

parallel lots of wheat were studied. The conditions under which these lots were produced were similar except for the nitrogen supply. The total nitrogen contents were widely different. Protein and non-protein nitrogen were determined, and these groups were further divided into their principal components. The results of the various analyses are presented in this paper. Some of the questions raised in this section are further discussed as the results are considered.

## Experimental

### *Material*

The material used in this study was Marquis wheat grown in water cultures during the summer of 1932. The seed was obtained from a single head row. It was soaked overnight, spread out on cheesecloth over water on May 26, and the seedlings set out in tanks on June 2. The tanks were each 30 by 30 by 8 in., and the tops each held 64 corks. Each cork supported one plant. The composition of the Hoagland's nutrient solution used in the tanks was as follows:—molar calcium nitrate, 3.9 cc.; molar potassium nitrate, 3.6 cc.; molar magnesium sulphate, 2.2 cc.; molar potassium dihydrogen phosphate, 1.1 cc.;  $\frac{1}{2}\%$  ferric tartrate, 1.0 cc.; water to one litre. Ferric tartrate was supplied every three days during the early stages of growth, and every second day after the plants were one month old. On June 24 the solutions were renewed in all tanks. On July 18 the plants began to head, and on July 19 the solutions were renewed in half the tanks but changed to a nitrogen-free solution in the others. The plants in the first three tanks had a supply of nitrate available until they were mature, and are hereafter designated "complete culture" plants. The plants in the other tanks had a supply of nitrate until they began to head, but from then on had no nitrogen supply. These plants are hereafter designated "limited nitrogen" plants.

Collection and analysis were started when the plants were 26 days old. Five plants were taken at each sampling. Four collections were made before the plants began to head, three between the time of heading and the start of rapid kernel formation, and five between this time and maturity. In the last five collections vegetative parts and kernels were analyzed separately.

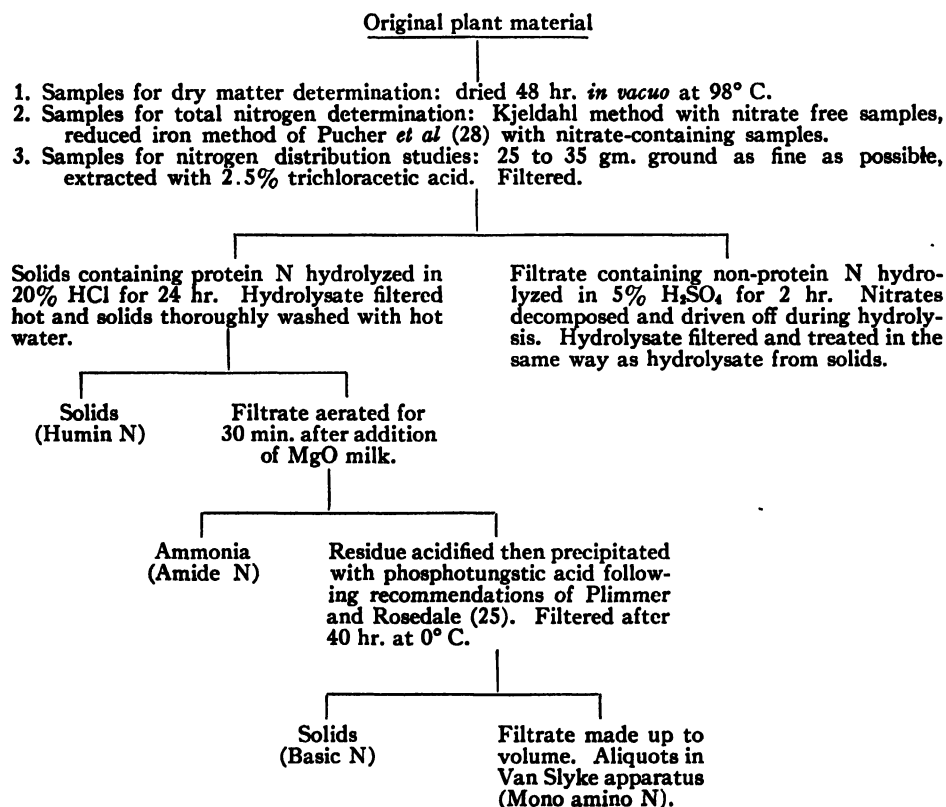
When the grain was mature, 64 plants of each lot were collected. The heads were counted and then threshed. The grain obtained was used in a number of supplementary experiments.

Unfortunately the light and temperature conditions could not be controlled as rigidly as was desired. The variations in size and weight of individual plants were great enough to decrease the value of the actual weights of various nitrogen compounds, as calculated from the analyses, so that these have only a relative significance. It is believed that the percentage values were little influenced by the variations in weight. In preliminary experiments it was found that the age rather than the size of the plant determined the nature of its nitrogen distribution.

## Methods

None of the methods described are entirely new, but the application of certain of them to the study of nitrogen distribution in green plants has not, as far as the writer knows, been attempted before. The general scheme of analysis is presented in the following outline. The details of those methods which have been modified, tested or newly applied are described in the notes.

### GENERAL SCHEME OF ANALYSIS



**Total nitrogen.** When nitrate nitrogen is present in material to be analyzed by the Kjeldahl method some of the nitrate will be reduced and recovered as ammonia. Some workers have assumed that adding a constant amount of sugar to the material to be analyzed will ensure at least comparable results, even if the nitrate is not completely recovered (22). To test this assumption, samples of pure potassium nitrate were subjected to the Kjeldahl method, while similar samples were added to nitrate-free ground wheat kernels and analyzed.

The pure nitrate was negligibly reduced by the method, but the presence of the organic matter of the wheat caused marked reduction. The results

of the experiment are presented in Table I. The ground wheat used contained 35.8 mgm. of nitrogen.

TABLE I  
RECOVERY OF NITRATE-N DURING KJELDAHL ANALYSES

Sample	N added as KNO <sub>3</sub> , mgm.	N recovered as NH <sub>3</sub> , mgm.	N due to reduced NO <sub>3</sub> <sup>-</sup> , -mgm.	% of NO <sub>3</sub> -N reduced
1	20.8	43.5	7.7	37.0
2	20.8	44.0	8.2	39.4
3	8.31	42.7	6.9	83.2
4	8.31	42.8	7.0	84.2
5	5.82	40.5	4.7	80.7
6	5.82	40.4	4.6	79.0
7	3.32	38.5	2.7	81.3
8	3.32	38.4	2.6	78.3

It was apparent that when the ordinary method was used, not more than 8 mgm. of nitrogen in the form of nitrate was reduced, and no matter how little nitrate was present, approximately 20% was lost during the determination. Adding sugar to the samples to be analyzed did not bring about increased recovery. Thus while the addition of sugar to all samples was satisfactory for the purposes of the study carried out by Newton and Brown (22), it would not be satisfactory in such a study as the present one if there were large variations in the amount of nitrate present in the material to be analyzed. It was found, however, that complete recovery of nitrate nitrogen, up to at least 50 mgm., was obtained when the reduced iron method of Pucher *et al.* (28) was used.

#### *Separation of Protein and Non-protein Nitrogen*

Considerable preliminary work was carried out before a satisfactory procedure was evolved for extracting the non-protein nitrogen from the plant material. It was first attempted to determine non-protein nitrogen, protein soluble, and protein insoluble, in dilute salt solution, but this was abandoned because it was almost impossible to get uniform extraction without first killing the cells. In common practice plant cells are killed by freezing or narcosis, but it has been shown that these treatments may cause some changes in the fractional distribution of nitrogen. Either method entails extra work which would make impossible the regular collection and treatment of any large number of samples unless there was considerable technical assistance available. There seems to be no definite physiological roles which can be ascribed to different proteins in the vegetative parts of plants, if indeed we are justified in assuming that different proteins exist. The writer decided that it would be better to extract the non-protein nitrogen with a protein precipitating agent, and study all of the protein together.



The protein precipitating agent selected must be simple to use, and should precipitate only proteins and not the less complex nitrogen compounds. The reagent selected was 2.5% trichloroacetic acid. Wasteneys and Borsook (38) found that it precipitated proteins and metaproteins from incomplete protein hydrolysates. Thomas (34) stated that it was not as selective as it was claimed to be because he found it precipitated peptones and proteoses. Despite these and other criticisms, it was found that Hiller and Van Slyke (14), who made studies of various protein precipitants, recommend the use of trichloroacetic acid where it is desired to precipitate the proteins but retain in the filtrate not only amino acids but also a maximum proportion of intermediate products.

A study of the precipitating action of trichloroacetic acid was made by adding sufficient of the reagent to solutions of various nitrogen compounds to bring the final concentration to 2.5%. The amounts of alanine, glutamic acid, arginine, asparagine, peptone and gelatin precipitated were all 0.0%. Proteins extracted from barley plants with water and dilute salt solutions and egg albumen were all more than 99.5% precipitated, or 100% within the experimental error of the Kjeldahl method. The substances studied do not represent all of the nitrogen compounds found in green plants, but they probably differentiate fairly well between the compounds active and inactive in translocation. This fact, together with the simple technique involved in the use of the reagent, seemed to justify its selection.

The technique finally evolved for the separation of protein and non-protein nitrogen provided for the simultaneous extraction of soluble compounds and precipitation of proteins. Samples of 25 to 35 gm. of green plant material were ground as fine as possible in a meat chopper. The chopper was rinsed with 2.5% trichloroacetic acid, the ground tissue and washings being collected in a beaker. Sufficient 50% trichloroacetic acid to compensate for the water in the plant material was added. The acid killed the cells rapidly. After standing for 20 min., as much of the liquid as possible was drained off and filtered. Any solids which had been transferred to the filter paper were returned to the beaker, and 50 cc. of 2.5% trichloroacetic acid added. After again standing for 20 min. the liquid was drained and filtered as before. The extraction was carried out four times, care being taken to stir the contents of the beaker after each addition of acid. It was found that additional extractions did not contain non-protein nitrogen compounds. The liquid from the four extractions was bulked and contained all the non-protein nitrogen, while the protein was retained in the solid material.

When kernels were analyzed, a 6-8 gm. sample of finely ground material was extracted with 2.5% trichloroacetic acid. The same procedure as outlined above for green tissues was followed, except that each extraction was followed by centrifuging before the extract was filtered.

The protein was not extracted from the other solids but was transferred directly to a 300-cc. Kjeldahl flask in which hydrolysis was carried out. Chibnall and Schryver (3) state that separation of protein from the other

solid materials of green plants must be obtained if the proteins are to be studied in detail. Hydrolysis of protein without extraction has been carried out by others in studying other problems. Blish (1) hydrolyzed flours and studied the amide nitrogen distribution. Grindley and Slater (13) hydrolyzed feedstuffs and studied various nitrogen fractions. The writer realized that the direct hydrolysis technique is definitely limited in its value for use in studying metabolic relations. Since it was impossible under the limitation of time and assistance to carry out a more detailed study, it was thought that valuable information could be obtained by utilizing this technique.

*Hydrolysis.* After the solid material had been hydrolyzed for 24 hr. in 20% hydrochloric acid, the volume of the hydrolysate was reduced to get rid of some of the excess hydrochloric acid. It was found that if the reduction of volume was carried slightly too far, some of the nitrogen produced from amide groups was recovered, not as ammonia, but in the basic fraction. If reduction was carried still further, the amount of ammonia was greater than it should have been, owing to splitting of amino groups from both basic and mono-amino acids. It was determined that the volume of the hydrolysate could be safely reduced to one-half the original, but that additional reduction was not always satisfactory. When replicate samples of wheat kernels were hydrolyzed and the hydrolysates evaporated down to different degrees, the following results were obtained.

TABLE II  
RECOVERY OF NITROGEN FRACTIONS FROM HYDROLYSATES OF WHEAT KERNELS

Treatment of hydrolysate	Amide N	Basic N	Mono-amino N
Volume not reduced	13.5	19.0	54.4
Volume reduced to $\frac{3}{4}$ original	13.4	19.0	54.6
Volume reduced to $\frac{1}{2}$ original	9.6	23.6	54.6
Volume reduced to remove practically all HCl	19.2	15.9	51.5

With hydrolysates of washed gluten, these effects were not noticed until the reduction of volume had been carried much further and would probably never interfere with an ordinary hydrolysate. The final increase in ammonia is more or less to be expected under the conditions of excessive reduction of volume. So far the writer has not been able to follow this subject any further.

In the hydrolysis of the non-protein nitrogen, sulphuric acid was used in preference to hydrochloric acid on the recommendation of Vickery and Pucher (37) who found that hydrolysis with hydrochloric acid in the presence of nitrates gave very high amide results.

*Humin nitrogen.* Humin nitrogen was taken to be the nitrogen left in the solids after hydrolysis. The amount of this fraction varies a great deal depending on the conditions under which hydrolysis is carried out. It was at one time thought that tryptophane was solely responsible for humin

formation (10, 11), but it has been shown that other amino acids, particularly the basic acids, contribute to the formation of this fraction (15, 29). It is believed (10) that absorption of ammonia by non-nitrogenous humins is not important in humin nitrogen formation, so the amide values would not be affected by variations in the amount of humin formed. Grindley and Slater (13) found that the amount of humin formed when feedstuffs were hydrolyzed was directly related to the amount of non-nitrogenous materials present in the feeds.

These facts were fully appreciated in carrying out the hydrolysis of protein in the presence of the other solids. It was realized that the humin value would not be a measure of any definite nitrogen fraction occurring in the plant. If, however, humin was formed at the expense of the basic amino acids this relation should be reflected in basic nitrogen results. It was believed that the objections based on the humin nitrogen formation were not sufficient to outweigh the advantages of the general method of attack.

**Amide nitrogen.** In determining amide nitrogen the method described by Plimmer and Rosedale (25) was followed except that magnesium oxide was used as the basic reagent for releasing ammonia. There have been many reagents recommended for this purpose, and there appeared to be no definite agreement as to which was the best. A survey of the literature showed that calcium oxide (7, 25), magnesium oxide (7, 24), a mixture of 5% sodium carbonate and 5% sodium chloride (33), and a phosphate buffer of pH 7.4 (23) were apparently all satisfactory. The buffer had been recommended for use in sewage analysis, and while it gave excellent results with solutions of individual compounds, it was unsatisfactory with hydrolysates which contained appreciable amounts of acid.

TABLE III

PER CENT RECOVERY AS AMMONIA OF NITROGEN IN  
NITROGENOUS COMPOUNDS

Nitrogen compounds	Basic reagents		
	5% $\text{Na}_2\text{CO}_3$ + 5% $\text{NaCl}$	$\text{MgO}$	$\text{CaO}$
Ammonium sulphate	98.6	99.3	99.2
Urea	3.5	0.0	0.6
Asparagine	1.2	0.2	0.0
Alanine	0.6	1.1	0.8
Glutamic acid	0.0	0.0	0.4

The other three reagents were tested with various compounds which might be present in the hydrolysate. The results are recorded in Table III.

None of these reagents caused much splitting of ammonia from the compounds studied, but the results for magnesium oxide and calcium oxide were better than for sodium carbonate plus sodium chloride.

The use of magnesium oxide or calcium oxide has been criticized on the grounds that any excess of these reagents makes the subsequent manipulation of the residue from the amide determination difficult. Plimmer and Rosedale recommend the careful addition of calcium oxide to avoid large excesses. The writer found that the excess of magnesium oxide which did not interfere

with subsequent manipulation could be much greater than that of calcium oxide. For this reason magnesium oxide was selected for use.

**Basic nitrogen.** In the determination of basic nitrogen the recommendations of Plimmer and Rosedale (25) were followed. No corrections were made for the solubility of the bases.

**Nitrate nitrogen.** When plant material containing nitrates was hydrolyzed, complete recovery of the total nitrogen was never obtained. It was thought that if the nitrates were completely lost during hydrolysis the difference between the total nitrogen, as determined by the reduced iron method, and the recovery in the hydrolysates might be taken as an indirect but accurate measure of nitrate content. To check the loss of nitrates during hydrolysis two experiments were carried out. In the first, 20.8 mgm. of nitrogen in the form of potassium nitrate was added to nitrate-free extracts containing organic matter and hydrolysis carried out in the usual way. The extracts contained 89.3 mgm. of nitrogen before the addition of the nitrate. In the second experiment 20.8 mgm. of nitrate nitrogen was hydrolyzed in the presence of sucrose. The results of both experiments are presented in Table IV.

TABLE IV  
LOSS OF NITRATE DURING HYDROLYSIS

Sample	Weight of T.N., mgm.	Weight of NO <sub>3</sub> -N, mgm.	Recovery of T.N., mgm.	Recovery of T.N., %	Nitrogen loss, mgm.
<i>Experiment 1</i>					
Extract	89.3	0	89.0	99.7	0.3
Extract	89.3	0	88.8	99.4	0.5
Extract + nitrate	110.1	20.8	89.0	80.8	21.1
Extract + nitrate	110.1	20.8	88.9	80.7	21.2
<i>Experiment 2</i>					
Sugar + nitrate	20.8	20.8	0.26	1.2	20.5
Sugar + nitrate	20.8	20.8	0.00	0.0	20.8

While hydrolysis was in progress, brown fumes could be distinctly seen escaping from the Kjeldahls in which hydrolysis was carried out. The experiments showed that the nitrate loss was complete.

In a later experiment using wheat plants which contained 38.6% of their total nitrogen in the form of nitrates and only 7.8% as organic non-protein nitrogen, the nitrates were completely lost during hydrolysis with sulphuric acid for two hours.

The indirect "difference" method of measuring nitrate nitrogen was checked against the direct method recommended by Vickery and Pucher (36). The results of both are recorded in Table V.

As a result of the various experiments it is considered that the "difference" method gives accurate and easily obtained values for nitrate nitrogen in any study where the nitrogen-containing compounds are hydrolyzed.

TABLE V  
NITRATE NITROGEN AS % OF TOTAL NITROGEN IN PLANT MATERIAL

Material	"Difference" method	Direct method of Vickery and Pucher
Roots from wheat plants one month old	30.4	31.5
Upper parts of wheat plants one month old	15.0	15.6
Upper parts of wheat plants two months old	9.5	10.1

### Results

The analytical results are presented in 11 tables and some of them are graphically depicted in nine figures. In each section, the results of analyses of vegetative parts are presented first and those of kernels immediately following. A general discussion is included in each section.

*Fresh and dry weights.* The fresh and dry weights of the vegetative parts of the plants during development are presented in Table VI. All the plants were grown under identical conditions until the time of heading, so the results given for complete culture plants apply to both lots until this stage.

Vegetative growth of the plants was almost complete before the nitrogen supply was varied. It is not surprising that the fresh and dry weights of the two lots were essentially the same. The variations which did occur were probably more the result of the sampling error than of real differences in the plants.

The rapid rise in both fresh and dry weights during the early stages was the result of production of new growth. The later decreases in fresh weight were the result of loss of moisture and translocation into the kernels. The decreases in dry weight were due to translocation of the reserves into the kernels.

TABLE VI  
FRESH AND DRY WEIGHTS OF VEGETATIVE PARTS PER PLANT

Date of collection	Age of plants, days	Fresh weight		Dry weight		Dry matter content	
		Compl.,* gm.	L.N.,† gm.	Compl., gm.	L.N., gm.	Compl., %	L.N., %
June 20	26	0.7	—	0.08	—	11.4	—
30	36	4.9	—	0.55	—	11.2	—
July 11	47	13.5	—	1.76	—	13.0	—
21	57	24.3	—	4.10	—	17.0	—
25	61	37.5	39.4	6.00	6.50	16.0	16.5
Aug. 1	68	27.0	30.8	5.13	6.04	19.0	19.6
10	77	28.9	27.3	6.46	6.00	22.5	22.0
18	85	21.0	23.7	4.71	5.68	22.4	23.9
25	92	23.0	20.8	5.67	5.54	24.6	26.6
Sept. 1	99	16.3	19.3	5.36	5.92	32.9	30.7
8	106	—	14.4	—	5.32	39.4	36.9
15	113	7.4	9.6	4.63	4.28	62.2	44.6
22	120	5.8	6.3	4.92	4.60	84.6	73.0

\* In some tables, "complete culture" plants are designated "Compl." for the sake of brevity.

† Similarly "limited nitrogen" plants are designated "L.N."

The percentage dry matter results are most clearly depicted in Fig. 1. The curves show that development was essentially the same in the two lots, although the limited nitrogen plants were slightly slower in maturing. The irregularities in the curves are probably the result of sampling errors.

The results of kernel analyses, presented in Table VII, show that the weight of dry matter in the kernels per limited nitrogen plant was consistently higher than the weight of kernels per complete culture plant. The difference in weight per 1,000 kernels was still more marked. The mature kernels weighed respectively 34.91 gm. and 29.14 gm. per thousand. The reasons for the differences noted are discussed in a later section of the paper.

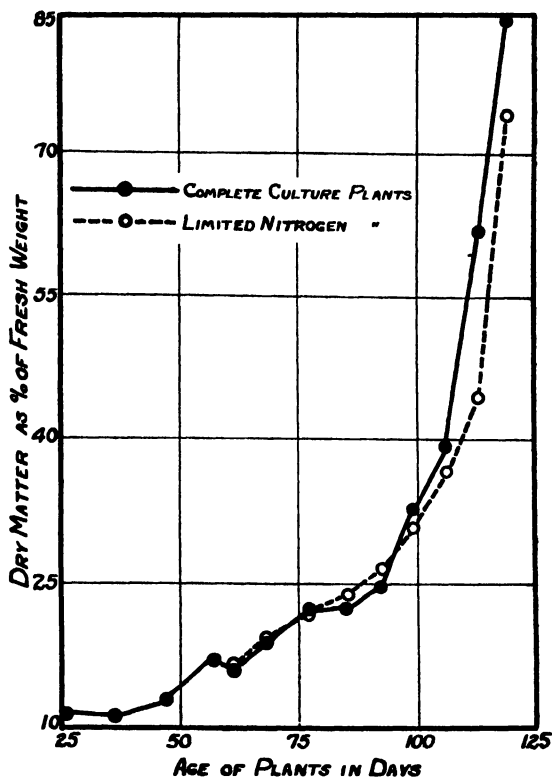


FIG. 1. Dry matter content of vegetative parts as related to age of plants and nitrogen nutrition.

TABLE VII  
FRESH AND DRY WEIGHT OF KERNELS PER PLANT

Date of collection	Time from heading, days	Fresh weight		Dry weight		Dry matter content	
		Compl., gm.	L.N., gm.	Compl., gm.	L.N., gm.	Compl., %	L.N., %
Aug. 18	30	4.24	4.33	1.62	1.72	38.2	39.7
25	37	5.17	5.12	2.40	2.49	46.4	48.6
Sept. 1	44	4.60	5.29	2.73	2.89	59.3	54.6
8	51	3.66	4.35	2.91	2.88	79.5	66.2
15	58	3.02	3.42	2.71	2.89	89.8	84.5

The percentage dry matter results are graphically presented in Fig. 2. The analyses were too few in number to yield satisfactory curves, but they show that the nature of kernel development was different from that of the development of vegetative parts. The kernels developed rapidly and regularly, and during development and desiccation large amounts of dry matter were translocated from the vegetative reserves. At maturity the kernels of complete culture plants made up 36.8%, and those of limited nitrogen plants 40.3%, of the total dry matter of the plants.

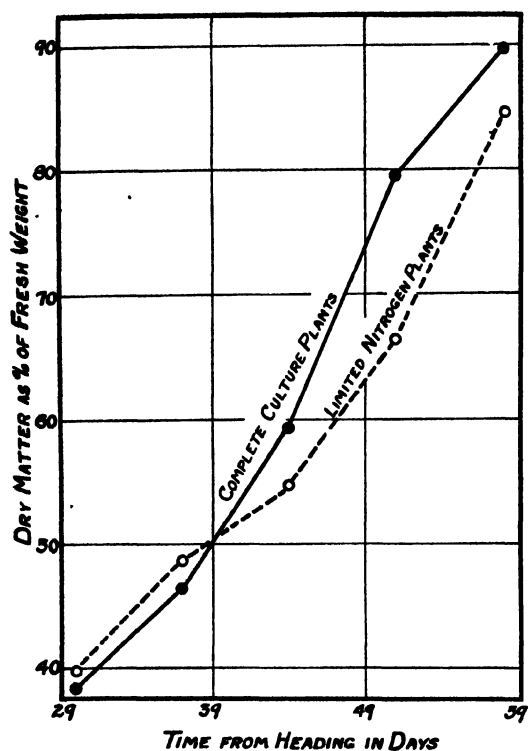


FIG. 2. Dry matter content of kernels as related to time from heading and nitrogen nutrition.

**Total nitrogen.** The results of the determinations of total nitrogen in vegetative parts of the plants are presented in Table VIII. The figures in columns 4 and 5 represent the total organic nitrogen in the materials. These values are included because, after the first collection, all the total nitrogen in limited nitrogen plants was organic, and the varying amounts of nitrate in the complete culture plants make it difficult to compare the total nitrogen results of the two lots. The comparison of total organic nitrogen results gives a much truer picture of differences than that of total nitrogen values. This distinction will be more clearly seen after the presentation of the protein and non-protein nitrogen results.

The percentage nitrogen relations may best be studied from Fig. 3. For the first 57 days the curves designated "complete culture

TABLE VIII

TOTAL NITROGEN OF VEGETATIVE PARTS OF PLANTS

Age of plants	Complete culture plants				Limited nitrogen plants*	
	Total nitrogen		NO <sub>3</sub> -free T.N.			
	As % of D.M.	Mgm. per plant	As % of D.M.	Mgm. per plant	As % of D.M.	Mgm. per plant
26	5.47	4.1	4.92	3.7	—	—
36	5.00	27.6	4.26	23.5	—	—
47	4.08	71.8	3.50	61.6	—	—
57	2.91	119.3	2.63	108.0	—	—
61	3.00	180.0	2.72	163.1	2.61	169.6
68	2.81	144.2	2.53	129.8	2.18	131.7
77	2.62	169.3	2.41	155.2	2.04	122.4
85	2.44	114.9	—	—	1.62	92.0
92	2.12	120.2	1.65	93.8	1.19	65.9
99	1.82	97.6	1.36	72.9	0.89	52.7
106	2.16	—	1.20	—	0.77	41.0
113	2.07	95.8	0.97	44.8	0.70	30.0
120	1.98	97.4	—	—	0.65	29.9

\* Total nitrogen and nitrate-free total nitrogen the same in limited nitrogen plants.

plants" serve for both lots. The initial rapid growth was accompanied by a marked fall in percentage of total nitrogen. These results agree with those of other workers who found that older tissues contain lower percentages of nitrogen than do new parts. The proportion of older tissues increases with age, so a fall in percentage nitrogen is to be expected. From the time the plants were 57, until they were 77, days old the heads were appearing. The relative proportion of old to new growth was changing less rapidly during this time, so the curves flatten out to some extent. During this time, also, the complete culture plants continued to absorb and reduce nitrates, so the decrease in percentage nitrogen was not as great as in the case of the limited nitrogen plants. When active translocation into the kernels began, both the percentage and weight of total nitrogen decreased rapidly. It is during this last stage of development that the inclusion of the nitrate in the total nitrogen values obscures most seriously the true picture of the nitrogen relations of the two lots of plants.

The results of total nitrogen analyses of kernels, presented in Table IX, show a definite upward trend in percentage nitrogen during the filling and ripening of the kernels. This trend was much more pronounced in the kernels from complete culture plants. The results agree in general with those obtained by McCalla and Newton (18) in a much

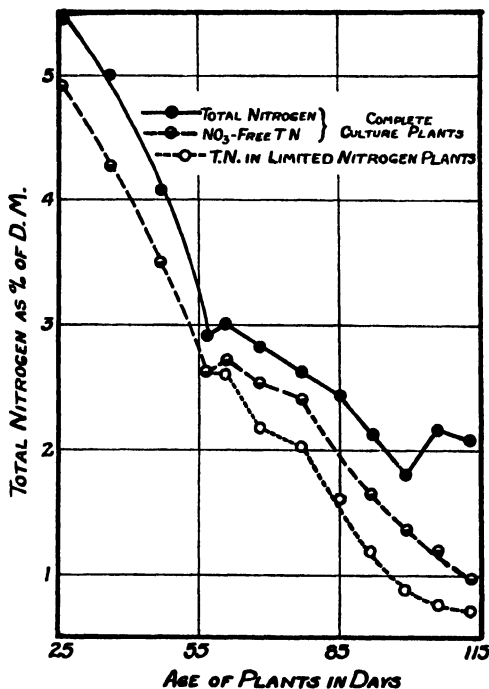


FIG. 3. Percentage total nitrogen of vegetative parts as related to age of plants and nitrogen nutrition.

TABLE IX  
TOTAL NITROGEN CONTENT OF KERNELS

Time from heading, days	Dry matter content		Total nitrogen as % of dry matter		Weight of total nitrogen per plant	
	Compl., %	L.N., %	Compl., %	L.N., %	Compl., mgm.	L.N., mgm.
30	38.2	39.7	3.22	2.58	52.1	44.4
37	46.4	48.6	3.40	2.48	81.6	61.7
44	59.3	54.6	3.68	2.66	100.5	76.8
51	79.5	66.2	3.86	2.82	113.0	81.2
58	89.8	84.5	4.26	2.88	115.6	82.9



more extensive study, and with those obtained by Woodman and Engledow (39). The value 2.88% is more or less common for Marquis wheat grown under favorable conditions, but the value 4.26% is extremely high. The results in Table IX show that the weight of total nitrogen was greater in complete culture kernels at every sampling than in the corresponding limited nitrogen kernels. This is in contrast to the dry matter results in which the reverse relation held.

It has already been mentioned in the introduction that, if it is available,

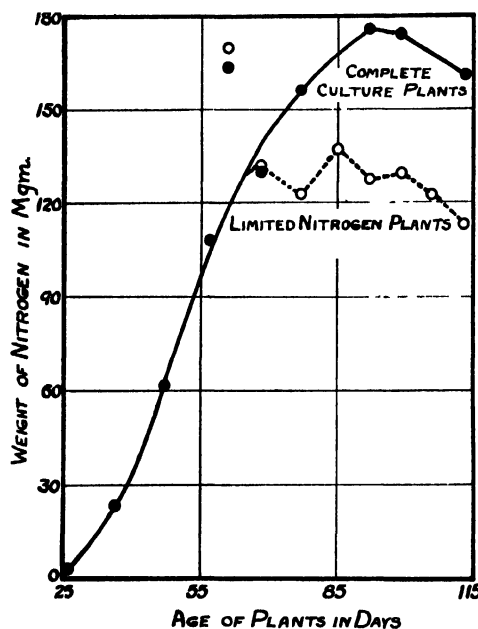


FIG. 4. Weight of total organic nitrogen per whole plant as related to age and nitrogen nutrition.

nitrate will be adsorbed and utilized by the plant for some time after heading. The weights of nitrate-free total nitrogen per whole plant are recorded in Table X, and are graphically presented in Fig. 4. It is obvious that synthesis of organic nitrogen continued for some time after heading. The final weight of organic nitrogen per whole complete culture plant was approximately 175 mgm., while that per whole limited nitrogen plant was approximately 130 mgm. Thus about 25% of the total organic nitrogen in the complete culture plants was synthesized after the plants had headed.

Despite the relatively large difference in amount of organic nitrogen synthesized by the plant, translocation into the kernel drained the vegetative reserves of complete culture plants almost as completely as those of limited nitrogen

TABLE X  
WEIGHT OF NITRATE-FREE NITROGEN PER WHOLE PLANT

Age of plants, days	Complete culture plants			Limited nitrogen plants		
	Veg. parts, mgm.	Kernels, mgm.	Total, mgm.	Veg. parts, mgm.	Kernels, mgm.	Total, mgm.
26	3.7	—	3.7	—	—	—
36	23.5	—	23.5	—	—	—
47	61.6	—	61.6	—	—	—
57	108.0	—	108.0	—	—	—
61	163.1	—	163.1	169.6	—	169.6
68	129.8	—	129.8	131.7	—	131.7
77	155.8	—	155.8	122.4	—	122.4
85	—	52.1	—	92.0	44.4	136.4
92	93.8	81.6	175.4	65.9	61.7	127.6
99	72.9	100.5	173.9	52.7	76.8	129.5
106	—	113.0	—	41.0	81.2	122.2
113	44.8	115.6	160.4	30.0	82.9	112.9

plants. In the final collection it was found that 71.5% of the organic nitrogen of the former, and 73.5% of that of the latter, was in the kernels. These results are in distinct contrast to the values 36.8% and 40.3% for the proportions of dry matter in the plants which were found in the kernels.

*Protein, non-protein and nitrate nitrogen.* The total nitrogen of the plants was resolved into protein, organic non-protein and nitrate nitrogen. The results of the group analyses of vegetative parts are reported in Tables XI and XII. The data in Table XI are graphically presented in Figs. 5 and 6, and those in Table XII in Fig. 7.

During the development of the plants from the early stages until translocation into the kernels began, the amount of nitrate nitrogen varied from 8.0 to 14.9% of the total nitrogen. The fluctuations were probably due to variations in the relative activity and efficiency of absorbing organs and reducing mechanism. During the last month of growth, however, nitrates accumulated in the leaves and stems, both percentage and weight of nitrate nitrogen increasing. The rise in percentage may be clearly seen in Fig. 5. This increase in nitrate was the result of continued absorption by the roots coupled with decreased, and finally discontinued, reduction in the plant tissues. The complete culture plants in the final collection contained in their

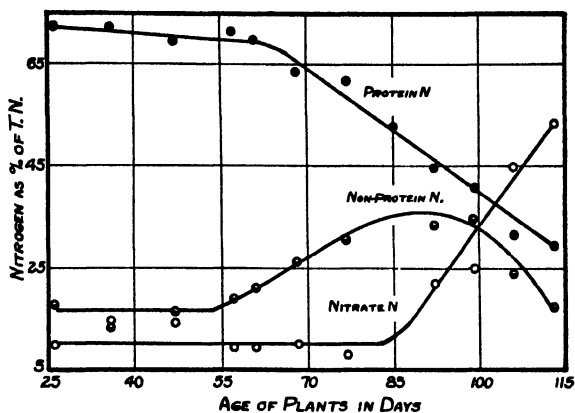


FIG. 5. Protein, organic non-protein and nitrate nitrogen in vegetative parts of complete culture plants as related to age.

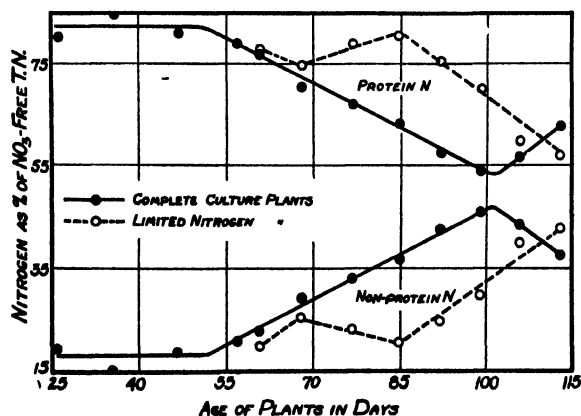


FIG. 6. Protein and organic non-protein nitrogen of vegetative parts as influenced by age of plants and nitrogen nutrition.

vegetative parts 50.9 mgm., or over 50% of their total nitrogen in the form of nitrates. This amount was greater than that found at any other time during development.

The influence of nitrate accumulation on the percentage protein and non-protein nitrogen as calculated on the basis of total nitrogen is clearly seen from the curves in Fig. 5. When these curves are compared with those in Fig. 6 it is at once apparent that calculating the protein and non-

TABLE XI  
PROTEIN, ORGANIC NON-PROTEIN AND NITRATE NITROGEN OF VEGETATIVE  
PARTS OF PLANTS

Age of plants, days	Complete culture plants					Limited nitrogen plants			
	Protein N		Organic non-protein N		Total organic N as % of T.N.	Nitrate N as % of T.N.	Protein N as % of T.N.*	Non-protein N as % of T.N.*	Total organic N as % of T.N.
	As % of T.N.	As % of NO <sub>3</sub> -free T.N.	As % of T.N.	As % of NO <sub>3</sub> -free T.N.					
26	72.2	80.2	17.8	19.8	90.0	10.0	—	—	—
36	72.0	84.6	13.1	15.4	85.1	14.9	—	—	—
47	69.5	81.0	16.3	19.0	85.8	14.2	—	—	—
57	71.5	79.0	19.0	21.0	90.5	9.5	—	—	—
61	69.8	77.0	20.8	23.0	90.6	9.4	77.8	20.0	97.8
68	63.4	70.5	26.5	29.5	89.9	10.1	74.9	25.4	100.3
77	61.6	67.0	30.4	33.0	92.0	8.0	79.0	23.2	102.2
85	52.7	63.3	—	36.7	—	—	80.5	20.7	101.2
92	44.8	57.4	33.2	42.6	78.0	22.0	75.3	24.7	100.0
99	40.5	54.0	34.5	46.0	75.0	25.0	69.9	29.9	99.8
106	31.4	56.8	23.9	43.2	55.3	44.7	59.9	40.0	99.9
113	29.4	62.7	17.5	37.3	46.9	53.1	57.2	42.8	100.0

\* Total nitrogen and nitrate-free total nitrogen the same in limited nitrogen plants.

protein nitrogen as percentages of the nitrate-free total nitrogen, rather than as percentages of the total nitrogen, gives the truer picture of the relations in complete culture plants. During the later stages of growth, the nitrate accumulation was greatest, so during this time the protein and non-protein relations of complete culture plants were in Fig. 5 most erroneously depicted in comparison with those of limited nitrogen plants (Fig. 6).

TABLE XII  
WEIGHT OF PROTEIN, ORGANIC NON-PROTEIN AND NITRATE NITROGEN IN  
VEGETATIVE PARTS PER PLANT

Age of plants, days	Protein nitrogen		Organic non-protein N		Total organic N		Nitrate N
	Compl., mgm.	L.N., mgm.	Compl., mgm.	L.N., mgm.	Compl., mgm.	L.N., mgm.	
26	3.0	—	0.7	—	3.7	—	0.4
36	19.7	—	3.6	—	23.6	—	4.1
47	49.9	—	11.7	—	61.6	—	10.2
57	85.3	—	22.7	—	108.0	—	11.3
61	125.6	131.9	37.4	33.9	163.0	165.0	16.9
68	91.4	98.8	38.2	33.4	131.4	131.7	14.6
77	104.3	96.7	51.5	28.4	155.8	125.1	13.5
85	60.6	74.1	—	19.0	—	93.1	—
92	53.8	49.6	39.9	16.3	93.7	65.9	26.4
99	39.6	36.8	33.8	15.8	73.4	52.6	24.5
106	—	24.6	—	16.4	—	41.0	—
113	28.2	17.2	16.8	12.8	45.0	30.0	50.9

Thus although protein and non-protein results were calculated on the basis both of total, and of nitrate-free total, nitrogen, the former will not be discussed further because it is impossible to compare them with the results for limited nitrogen plants which contained no nitrate. The protein and non-protein organic nitrogen are considered to equal 100% of the nitrogen active in metabolism at the time the sample was collected.

As in the previous sections, the results for complete culture plants serve for both lots for the first four collections. During these first 57 days the percentage protein nitrogen remained fairly constant at approximately 80% of the total organic nitrogen, but the weight increased up to 85.3 mgm. per plant. The weight of organic non-protein nitrogen increased to 22.7 mgm. Growth was regular during this time as new materials were being continually produced. When heading became general the production of new tissue lessened. The percentage protein in vegetative parts of complete culture plants decreased while that in limited nitrogen plants increased. Fig. 7, however, shows that the weight of protein nitrogen was almost the same in the two lots at all stages of development, and that the organic nitrogen synthesized from nitrates in the complete culture plants after heading was not converted to protein, but accumulated in the form of non-protein compounds. Between the time the heads began to appear and the time they began to fill, some of the non-protein nitrogen was utilized in the completion of vegetative development.

In complete culture plants the continued production of organic non-protein nitrogen more than supplied the amount needed in the final vegetative development, so during this stage of development the percentage of organic non-protein nitrogen increased and the percentage of protein nitrogen consequently decreased. In limited nitrogen plants the nitrogen used in the final vegetative development was not replenished from external sources, so the percentage protein nitrogen in the whole plants increased, although there was no increase in weight of total nitrogen. Thus while the actual amount of protein in each of the two lots was about the same, the percentage in the complete culture plants decreased, and that in the limited nitrogen plants increased.

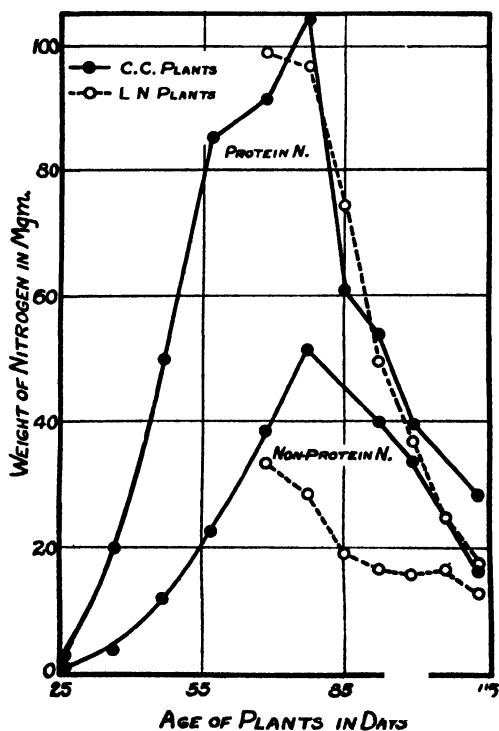


FIG. 7. Weight of protein and organic non-protein nitrogen of vegetative parts as influenced by age of plants and nitrogen nutrition.

When the plants were fully headed and translocation into the kernels began, both the percentages and weights of protein in both lots decreased. The percentage non-protein nitrogen of necessity increased, but the weight decreased. During the development of the kernels, the protein reserves in vegetative parts were hydrolyzed and the degradation products utilized in the synthesis of proteins in the kernels. The increase in percentage non-protein nitrogen in vegetative parts was the result of degradation of the protein reserves at a faster rate than the products were translocated to the kernels. The final increase in percentage protein in complete culture plants was accompanied by a continued decrease in its weight. During the last 10 days the degradation of protein reserves apparently slowed down to a greater extent than did the translocation of materials into the kernels. The removal of the non-protein compounds caused an increase in percentage protein while the total amount was still decreasing. The final rise was not noted in the percentage protein of limited nitrogen plants.

Table XIII presents the results of group analyses of the kernels. Nitrate nitrogen was undemonstrable in the kernels at any time during development. All of the nitrogen was probably moved into the kernels in the form of non-protein compounds, but the amount of these in the kernel was low at all stages studied. It was previously found by McCalla and Newton (18) that non-protein nitrogen in forming kernels made up about 38% of the total nitrogen, but that as soon as rapid filling began, the non-protein nitrogen decreased rapidly. In the present study, the percentage non-protein nitrogen decreased slightly during the filling of the kernels while the weight increased slightly. There was little difference in percentage protein and non-protein nitrogen in the two lots, but the kernels from complete culture plants contained a much greater weight of protein than did those from limited nitrogen plants.

The general conclusion to be drawn from the protein-non-protein results is that modifying the nitrogen nutrition of wheat plants affects the distribution of protein and non-protein nitrogen compounds in the vegetative parts, but

TABLE XIII  
PROTEIN AND NON-PROTEIN NITROGEN OF KERNELS

Time from heading, days	Dry matter content		Protein nitrogen				Non-protein nitrogen				Recovery of total nitrogen	
	Compl., %	L.N., %	As % of total N		As weight per plant		As % of total N		As weight per plant		Compl., %	L.N., %
			Compl., %	L.N., %	Compl., mgm.	L.N., mgm.	Compl., %	L.N., %	Compl., mgm.	L.N., mgm.		
30	38.2	39.7	92.4	89.0	48.1	39.5	7.8	9.0	4.1	4.0	100.2	98.0
37	46.4	48.6	93.2	89.5	76.0	55.2	6.6	9.6	5.4	5.9	99.8	99.1
44	59.3	54.6	94.4	91.3	94.9	70.1	5.9	8.7	5.9	6.7	100.3	100.0
51	79.5	66.2	94.4	93.2	106.7	75.7	5.8	6.3	5.6	5.1	100.2	99.5
58	89.8	84.5	93.5	93.6	108.1	77.9	6.6	6.8	7.6	5.7	100.1	100.4

these effects are not carried over to the kernels. The only significant difference in kernels resulting from the limited nitrogen supply of plants is in the total weight of protein produced.

*Protein and non-protein fractions.* The protein and non-protein nitrogen compounds were hydrolyzed, and humin, amide, basic and mono-amino nitrogen fractions determined separately. The results of the determinations of protein fractions in vegetative parts are recorded in Table XIV, those of non-protein fractions in vegetative parts in Table XV, and those of both protein and non-protein fractions of kernels in Table XVI. Fig. 8 graphically presents some of the results recorded in Table XV, and Fig. 9 some of those in Table XVI.

The discussion of these results is more or less restricted to the differences due to nitrogen nutrition, although some other features are incidentally mentioned. Some of the results will be discussed more fully in a subsequent paper.

Table XIV shows that while the results of fractional analyses of proteins were somewhat irregular during the development of the vegetative parts, there were no distinct differences in the results of the analyses of the two lots of plants. Apparently the variation in nitrogen supply did not affect the distribution of fractions in the proteins formed. This is not at all surprising since it has already been shown that the variation in nitrogen supply did not affect the total amount of protein. During the development of the vegetative parts

TABLE XIV  
FRACTIONAL ANALYSES OF PROTEIN IN VEGETATIVE PARTS OF PLANTS

Age of plants, days	Humin N		Amide N		Basic N		Mono-amino N		Recovery	
	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %
26	7.2	—	8.6	—	32.6	—	52.9	—	101.3	—
36	11.2	—	8.1	—	32.2	—	49.8	—	101.3	—
47	9.0	—	5.7	—	36.0	—	48.3	—	99.0	—
57	15.8	—	5.0	—	32.1	—	53.6	—	106.5	—
61	15.4	14.8	4.4	4.3	31.6	32.7	46.5	45.6	97.9	97.4
68	15.5	15.7	5.1	3.1	32.1	31.0	51.4	49.5	104.1	99.3
77	13.5	12.4	5.8	6.7	32.2	29.8	51.6	55.9	103.1	104.8
85	14.2	—	6.2	9.2	28.4	30.1	52.4	50.0	101.2	—
92	21.6	19.0	4.4	3.2	28.0	27.5	48.8	50.1	102.8	99.8
99	13.8	19.6	4.3	5.4	26.8	24.0	54.2	51.1	99.1	100.1
106	18.5	20.0	7.2	5.0	26.4	18.3	39.5	56.2	91.6	99.5
113	19.7	19.8	7.4	5.0	20.7	24.2	44.9	34.0	92.7	83.0

of both lots of plants, the percentage humin nitrogen increased irregularly. This is related, as indicated in the section on methods, to the gradually increasing proportions of non-nitrogenous material shown by the decreasing values for percentage total nitrogen. The increase in humin was accompanied by an irregular decrease in basic nitrogen, which lends support to the belief that it is at the expense of the basic amino acids that the humin is formed.

TABLE XV

FRACTIONAL ANALYSES OF NON-PROTEIN NITROGEN IN VEGETATIVE PARTS OF PLANTS

Age of plants, days	Humin N		Amide N		Basic N		Mono-amino N		Recovery	
	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %
26	1.8	—	14.7	—	18.8	—	30.0	—	65.3	—
36	2.6	—	10.5	—	23.0	—	29.0	—	65.1	—
47	1.8	—	8.8	—	29.9	—	29.4	—	69.9	—
57	2.0	—	8.2	—	30.0	—	30.2	—	70.4	—
61	1.8	2.0	8.4	6.5	27.6	27.9	29.2	32.2	67.0	68.6
68	2.2	2.6	9.4	8.6	27.5	25.6	30.3	31.9	69.4	68.7
77	2.0	2.8	9.3	8.9	25.8	27.8	31.0	31.2	68.1	70.7
85	3.4	3.8	12.2	7.3	41.3	37.3	35.0	39.4	91.9	87.8
92	2.7	5.5	10.5	8.0	—	45.4	26.0	35.3	—	94.2
99	3.2	6.7	18.2	8.5	42.6	42.4	33.4	38.2	97.4	95.8
106	2.8	4.5	20.6	14.9	50.6	49.6	31.1	26.2	105.1	95.2
113	5.7	5.0	28.2	19.3	46.9	45.8	26.2	25.8	107.0	95.9

The results in Table XV show that varying the nitrogen supply did not affect the distribution of basic and mono-amino fractions of the non-protein

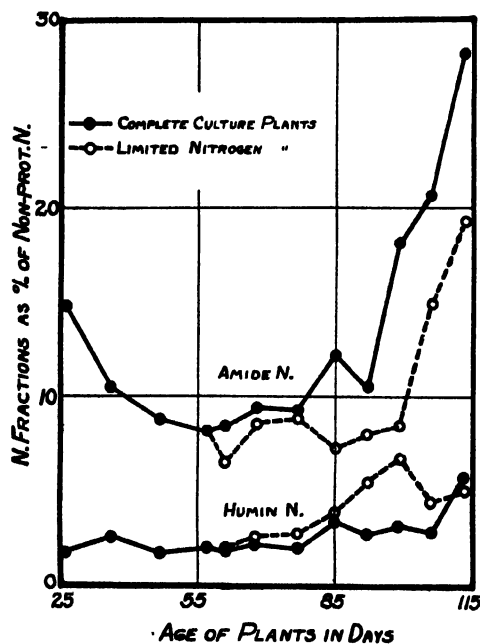


FIG. 8. Amide and humin fractions of non-protein nitrogen of vegetative parts as influenced by age of plants and nitrogen nutrition.

that in complete culture plants. Apparently the reduced nitrate in the latter was more readily converted to amide than to any other nitrogen fraction.

No mention has been made of ammonia nitrogen in the tables or discussion. Many workers in the field of nitrogen metabolism have believed that reduced

nitrates first make their presence felt in increased ammonia nitrogen content, but usually the amounts of ammonia reported have been very small (26). It has already been pointed out that some investigators have been unable to demonstrate the presence of ammonia in green plants. In the present study it was impossible to detect ammonia at any stage of the plant's development except during the first few days of kernel development. Even during this time the amounts were so small that they have been neglected in this discussion.

The results of the fractional analyses of the protein in kernels are most easily visualized from Fig. 9. The humin nitrogen of both lots decreased as the percentage total nitrogen increased, but the only distinct differences in response to nutritional treatment were in the amide and mono-amino fractions. As in the results for vegetative parts, so in the kernel protein results, the amide nitrogen was higher in complete culture than in limited nitrogen plants at all collections. The amide of the protein in both lots showed a fairly regular increase as the kernels developed. This was probably owing to gradually increasing proportions of the gluten proteins. Blish (1) found that the higher the proportion of gluten protein the higher the amide content of the mixed proteins of flour. This probably explains why the protein in complete culture kernels was at all times higher in its amide content than that in limited nitrogen kernels, as it is shown in the next section that the latter contained a smaller proportion of gluten proteins than the former.

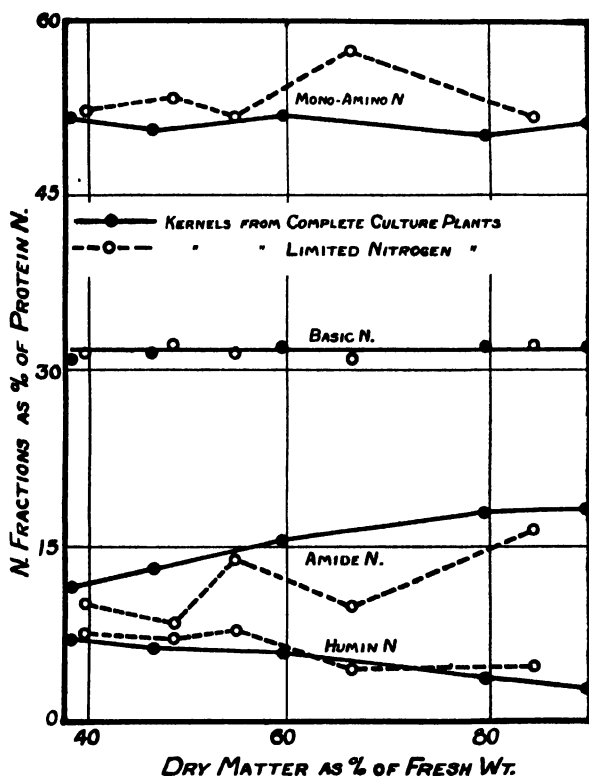


FIG. 9. Fractions of protein nitrogen of kernels as influenced by age of plants and nitrogen nutrition.

There was a very striking relation between the amide and mono-amino fractions of the protein in limited nitrogen kernels. The irregularities of amide values were compensated for by irregularities, in the opposite direction, of mono-amino values. Just why these irregularities should have occurred is not clear.



TABLE XVI  
FRACTIONAL ANALYSES OF NITROGEN IN KERNELS

Time from heading, days	Dry matter		Humic N		Amide N		Basic N		Mono-amino N		Recovery T.N.	
	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %	Compl., %	L.N., %
<b>Protein fractions</b>												
30	38.2	39.7	7.2	7.5	11.5	10.1	31.0	31.4	51.6	52.2	101.3	101.2
37	46.4	48.6	6.5	7.2	13.1	8.6	31.5	32.3	50.5	53.3	101.6	101.4
44	59.3	54.6	6.0	8.0	15.5	14.0	32.0	31.5	52.2	51.6	105.7	105.1
51	79.5	66.2	3.9	4.5	17.9	10.0	32.2	31.0	50.0	57.0	104.0	102.5
58	89.8	84.5	2.9	4.1	18.1	16.4	32.1	32.2	50.9	51.6	104.0	104.3
<b>Non-protein fractions</b>												
30	38.2	39.7	3.0	2.2	13.3	12.8	43.4	38.0	41.1	48.0	100.8	101.0
37	46.4	48.6	2.2	2.6	15.6	15.1	37.9	41.0	44.8	40.2	100.5	98.9
44	59.3	54.6	2.5	1.5	16.4	13.4	31.6	36.8	35.8	42.6	86.3	94.3
51	79.5	66.2	3.4	1.8	14.2	14.4	37.2	36.6	20.8	30.2	75.6	83.0
58	89.8	84.5	3.3	2.4	17.0	13.5	28.6	32.1	19.5	22.0	68.4	70.0

The results of the fractional analyses of non-protein compounds cannot be considered important. The amounts of non-protein nitrogen were so small that the basic and mono-amino results cannot be considered at all accurate. The amide results agree with the others discussed, in that the values for amide in limited nitrogen plants were always lower than corresponding values for amide in complete culture plants.

**Supplementary results.** When the wheat was fully mature 64 plants of each lot were collected from tanks which had not been sampled during the growing period. These plants were in every way comparable to those of the final collection of the main series except that the latter were collected from a tank from which most of the other plants had been removed in the course of previous collections. The supply of nitrate to the last collection of main series plants was probably greater than that to the plants collected from the full tank, and this, in turn, probably accounts for the lower total nitrogen content in the kernels from the latter. The data on yield, weight per thousand kernels, etc., of the 64 plants are presented in Table XVII.

TABLE XVII  
YIELD, WEIGHT AND GRADE OF MATURE KERNELS

Sample	No. of heads from 64 plants	Grade	Weight of dry matter		Number of kernels	
			From 64 plants, gm.	Per 1,000 kernels, gm.	From 64 plants	Per head
Complete culture	406	3°	173.7	29.14	5,960	14.7
Limited nitrogen	406	1 Hard	182.3	34.91	5,290	13.0

The formation of heads was obviously complete before the limited nitrogen plants were transferred from the complete culture solution. The number of kernels per head was greater in the complete culture plants, but the total yield was lower than that of the limited culture plants, owing to the difference in weight per 1,000 kernels. These differences in total yield and weight per 1,000 kernels were not sufficient to compensate for the high total nitrogen content of the complete culture kernels, and these gave the higher yield of total nitrogen and also contained more nitrogen per 1,000 kernels (Table XVIII).

TABLE XVIII  
TOTAL NITROGEN CONTENT OF MATURE KERNELS

Sample	Total nitrogen content, %	Weight per 1,000 kernels		Weight per 64 plants	
		Dry matter, gm.	Nitrogen, gm.	Dry matter, gm.	Nitrogen, gm.
Complete culture	3.74	29.14	1.090	173.7	6.10
Limited nitrogen	2.90	34.91	1.012	182.3	5.29

The high total nitrogen content of the complete culture kernels was accompanied by shrivelling to such an extent that, while the limited nitrogen kernels graded No. 1 hard, they graded No. 3 Northern. The shrivelling must have been the result of translocation of materials in insufficient quantities to completely fill the seed coat developed. It will be recalled that, while the kernels of limited nitrogen plants made up 40.3% of the total dry matter, those of complete culture plants made up but 36.8%. It seems reasonable to assume that the deficiency in translocation was related to the relatively large amounts of non-protein nitrogen elaborated in the vegetative parts of the plants after the limited nitrogen plants had been removed from the complete solution. If these non-protein compounds were produced, as seems likely, from a combination of reduced nitrate with soluble carbohydrates, it seems quite probable that the amount of these soluble carbohydrates available for direct translocation into the developing kernels would be seriously reduced. Sugar determinations made about three weeks after the time of heading showed that the vegetative parts of the limited nitrogen plants contained more than twice as much sugar as did those of the complete culture plants. It seems almost certain that the wheat plants in both lots synthesized carbohydrates to a very small extent after the heads were fully formed, so the soluble carbohydrates needed to produce the extra non-protein nitrogen must have come from the supply which would normally have been translocated to the kernels. This is essentially in agreement with Gericke's belief (8) that the ill-effects of overfeeding on wheat may be overcome by prolonged growth. Probably under the conditions of prolonged growth, carbohydrates would be produced until a much later stage of development than was the case in this study.

The distribution of protein and non-protein nitrogen and the fractions of these groups was essentially the same as in the final collection of the main series, so there is nothing to be gained from including the detailed results.

It was stated in the discussion of amide results that the limited nitrogen kernels contained a smaller proportion of their total nitrogen in the form of gluten proteins than did the complete culture kernels. Samples (25 gm.) of finely ground kernels were used in the ordinary washed gluten determination. During the washing, all soluble compounds and most of the bran particles were washed from the gluten along with the starch. Small samples of the gluten were used for the determination of total nitrogen. The remainder of the gluten was cut into small pieces, placed in a 100-cc. centrifuge tube and shaken mechanically with 70% alcohol for half an hour. The contents of the tube were centrifuged, and the supernatant liquid poured off. Other portions of alcohol were added and the extraction repeated a sufficient number of times to remove the soluble protein. Dilute sodium hydroxide was then added to the protein remaining in the tube, most of which was taken up by the alkali. The solids left were found to be bran particles and were discarded.

Aliquots of the alcohol and alkali extracts were taken for total nitrogen determinations. The relations between the various nitrogen groups studied are given in Tables XIX and XX.

TABLE XIX  
NON-GLUTEN NITROGEN IN MATURE KERNELS

Sample	Non-protein N as % of T.N.	Non-gluten protein N as % of T.N.	Total non-gluten nitrogen	
			As % of T.N.	As % of dry matter
Complete culture kernels	6.7	13.8	20.5	0.77
Limited nitrogen kernels	6.2	19.3	25.5	0.74

TABLE XX  
GLUTEN NITROGEN IN MATURE KERNELS

Sample	Weight of wet gluten, gm.	Alcohol soluble gluten		Alkali soluble gluten		Total gluten N	
		As % T.N.	As % gluten N	As % T.N.	As % gluten N	As % T.N.	As % dry matter
Complete culture kernels	22.2	40.6	51.1	38.9	48.9	79.5	2.97
Limited nitrogen kernels	14.7	38.1	51.1	36.4	48.9	74.5	2.16

When the gluten samples were washed it was impossible to tell the two apart. The color and texture were of high quality. The difference in weight of gluten reflected the difference in total nitrogen content of the wheat.

The non-gluten nitrogen of complete culture kernels expressed as a percentage of the total nitrogen was 5% lower than that of limited nitrogen kernels. When expressed as percentages of the dry matter there was little difference in the two lots. When these results are compared with those for gluten nitrogen it is at once apparent that the effect of the large supply of nitrogen to the complete culture plants was on the quantity of gluten nitrogen and not on that of the non-gluten. It would appear that the amount of nitrogen in what may be called the structural parts of the kernel is much more independent of the nitrogen nutrition of the wheat plant than is the amount in the endosperm.

Despite the fact that the amount of gluten nitrogen was influenced by the nitrogen nutrition, the proportion of alcohol-soluble to alkali-soluble gluten nitrogen was the same for both lots of kernels. The value 0.49 for the ratio of alkali-soluble protein to the alcohol-soluble protein plus the alkali-soluble protein was the same as that obtained by Grewe and Bailey (12) for several of the hard red spring wheat samples they studied. These workers found values as low as 0.41, however, for some of the samples tested, and the writer found that the value for a sample of Little Club wheat was 0.38. Thus, while the nutritional variation did not affect the distribution of the proteins of the two samples examined in this study, other factors, such as variety or type, did.

It is fully realized that the method used in separating the components of the gluten is empirical in nature. As Sandstedt and Blish (32) point out in their summary of our knowledge of these proteins, the amount of protein extracted by the alcohol depends on so many factors that it is difficult to standardize any such technique as has been employed in the present study. It has become more and more apparent during the last few years that the so-called gliadin and glutenin are not definite individual proteins, combinations of which make up the gluten. If, however, the proportions of the proteins making up the gluten, as determined by extraction under identical conditions, are exactly the same, as they were in this case, then it seems reasonable to assume that the two samples of gluten were the same.

Additional evidence for this belief is found in the results of the fractional analyses of the alcohol and alkali soluble gluten, presented in Table XXI.

TABLE XXI  
FRACTIONAL ANALYSES OF GLUTEN PROTEINS

Fraction	Alcohol soluble		Alkali soluble	
	Compl., %	L.N., %	Compl., %	L.N., %
Humin	0.7	0.7	2.8	2.3
Amide	20.6	20.3	10.0	10.0
Basic	16.6	16.3	22.4	22.2
Filtrate from basic	62.2	62.4	64.2	64.8
Recovery	100.1	99.7	99.8	99.3

These results do not agree very well with those for prepared gliadin and glutenin samples (4, 5), but it must be remembered that the whole of the gluten, and not just fractions of it, was analyzed. Cook (4) gives results showing that successive fractions of gluten proteins, precipitated from a urea dispersion with increasing concentrations of magnesium sulphate, contained amounts of arginine which indicated that they were made up of mixtures of gliadin and glutenin, the proportion of glutenin decreasing, and that of gliadin increasing, as the concentration of magnesium sulphate increased. Sandstedt and Blish (32) believe that the protein system of gluten is much more complex than has been believed and that the properties of the fractions going from the most, to the least, soluble show gradual changes.

If there was any marked difference between the gluten proteins of complete culture and limited nitrogen kernels, there should have been differences in the fractional analyses. Any differences which do occur are too small to be significant. The results for amide content of the alkali soluble protein are very low, but this has been shown to be due to the effect of alkali on the protein. Blish and Sandstedt (2) found that even short exposure to alkali reduced the amide content of glutenin in direct proportion to the strength of the alkali. The results show, however, that the effect was the same on both lots of protein.

### Discussion

The results reported in this paper are concerned with the effects of varying nitrogen nutrition on one variety of wheat. They are therefore limited in scope, and general conclusions cannot be too freely drawn. There was insufficient material to carry out milling and baking tests so the final measure of quality, as usually determined, could not be made.

There was a distinct difference in the quantitative distribution of non-protein nitrogen compounds in the vegetative parts of the plants studied. This difference in quantity of non-protein nitrogen was reflected in the total nitrogen content of the kernels produced by the plants. In the kernels from the plants whose supply of nitrogen was limited the total nitrogen content was 2.90%, while in the kernels from plants whose supply was unlimited it was 3.74%. The excess total nitrogen in the latter kernels was all in the form of gluten nitrogen, the non-gluten nitrogen being unaffected by the nutrition of the plants.

There were differences in the percentage composition of the two lots of kernels due to the difference in amounts of gluten, but there were no differences in the chemical composition of the gluten proteins. There were no differences in the physical nature of the glutens in so far as it was possible to tell from the studies made.

It seems safe to say, therefore, that in such ways as it has been possible to measure quality, there was no essential difference between the two lots of kernels except that which might result from a difference in the quantity of gluten. Differences do occur in the quality of the grain from various samples

of the same variety of wheat grown under varying field conditions. From the results of this study, these differences appear to be due to some factor or factors other than nitrogen nutrition. Gericke's recent work (9) tends to support the conclusion of some cereal chemists that the differences depend upon factors affecting the physical state of the gluten, but it is by no means proved that other differences may not be important.

A study of the chemical composition of wheat samples grown in water cultures varied with respect to elements other than nitrogen is now under way. It is hoped that this study will yield some definite results on the relation of chemical and physical differences in grain to the nutritional treatment.

### Acknowledgments

The writer wishes to thank Professor D. R. Hoagland of the University of California under whose direction the work was carried out, and Dr. R. Newton, Director of the Division of Biology and Agriculture, National Research Council of Canada, for their interest and assistance during the time the project was under way and for criticism of the manuscript.

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# A FURTHER NOTE ON CATALASE ACTIVITY AS A MEASURE OF SEED VIABILITY<sup>1</sup>

By C. W. LEGGATT<sup>2</sup>

## Abstract

Application of statistical methods to the results of an investigation previously published has shown that viability, in the case of common wheat, may be estimated fairly closely from a determination of total and thermostable catalase; a determination which may be completed within the course of a few hours as compared with the regular germination test requiring 12 days. Much work is yet required, however, to put this method on a practical routine basis.

It is believed that some further light is thrown upon the biochemistry of ripening in the wheat kernel.

In 1929 the present writer published a paper (1) reporting the results of a study to ascertain the relation, if any, between catalase activity and viability, using wheat seed of known history as material. It was shown that catalase is present in two conditions, thermostable and thermolabile—that there was a relation between the ratio thermostable: total catalase and the viability but that this relation was markedly affected by the amount of total catalase present, which in turn appeared to vary directly with the immaturity of the sample. The following statement was made under "Discussion" (1, p. 108):—

"This method appears to be capable of being developed into a useful accessory to the germination test, but a considerable amount of further study is required. . . . ."

"Relative degrees of immaturity of the seed introduce a complication which may require the introduction of a correction factor in order that all samples may be compared on an equal basis."

At the time that this was written, the writer had no knowledge of the theory of partial correlation. Recently, while looking over the paper in another connection, it occurred to him that the data reported in Table 13, page 108 (and reproduced here as Table I), might repay examination by this method.

This examination has given the following results:—

Correlations of zero order:

$$r_{GR} = +.85 \quad r_{GC} = +.01 \quad r_{RC} = -.41$$

Partial correlations of first order:

$$r_{GR.C} = +.94 \quad r_{GC.R} = +.75 \quad r_{RC.G} = -.79$$

Where G = germination, R = catalase ratio, C = total catalase.

(Value of  $r$  necessary for 5% level of significance = 0.58.)

These correlations of the first order are distinctly high.

<sup>1</sup> Original manuscript received August 28, 1933.

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TABLE I

SAMPLES SHOWING RELATIVELY HIGH AND LOW CATALASE RATIOS, TABULATED SEPARATELY

Sample	Germination	Ratio	Total catalase	Sample	Germination	Ratio	Total catalase
Samples showing high catalase ratios				Samples showing low catalase ratios			
D-16-20*	8	32.4	8.01	D-16-20*	8	32.4	8.01
65-1997	22	39.6	6.62	D-11-21	34	37.2	14.98
B-11-21	36	43.1	5.75	C-11-21	65	40.8	10.94
65-6756B	73	49.3	6.80	C-12-21	71	43.5	10.55
65-6756A	77	51.7	7.56	D-7-20	97	44.9	14.51
65-6501	80	53.9	6.21				
65-6883	87	54.3	8.20				
1	95	52.1	7.78				
A-16-20	100	53.0	5.83				

\* Sample D-16-20 has here been listed twice, since it appeared to occupy a position intermediate between the two groups. The values, of course, have only been taken once in the calculations.

It appears that for a given amount of total catalase, there is a high positive correlation between germination and the catalase ratio; for a given catalase ratio there is a fairly high positive correlation between germination and total catalase; while for any given percentage of germination high catalase ratios tend to be associated with low amounts of total catalase and *vice versa*.

The fact that immaturity is accompanied by high total catalase content which, in turn, is associated with low catalase ratios, would indicate that immature seeds have a greater proportion of thermolabile catalase.

The following picture is tentatively suggested. During the ripening of the seed, the thermolabile catalase (Loew's  $\beta$  catalase? (2)) becomes condensed to the thermostable condition. Since the proportion of thermostable catalase to total catalase decreases with decreasing viability we may depict, as one of the processes accompanying loss of viability, either a gradual reconversion of the thermostable catalase into the thermolabile condition or a destruction of the thermostable catalase directly or a combination of both processes.

The regression equation expressing germination in terms of the catalase ratio and total catalase derived from the statistics reported above is:—

$$G = 4.38 R + 4.21 C - 172.5 \pm 10.2$$

In Table II the germination figures predicted by the use of this equation and the observed values are given.

TABLE II  
GERMINATION OF SAMPLES OF WHEAT USED IN THIS STUDY.  
PREDICTED AND OBSERVED VALUES

Sample	Germination		Sample	Germination	
	Predicted	Observed		Predicted	Observed
D-16-20	3.1	8	65-6756A	85.8	77
65-1997	28.8	22	65-6501	89.8	80
D-11-21	53.5	34	65-6883	99.9	87
B-11-21	40.6	36	1	88.5	95
C-11-21	52.3	65	D-7-20	85.2	97
C-12-21	62.5	71	A-16-20	84.2	100
65-6756B	72.1	73			

The agreement is quite good for so small a number of observations, all values falling within a range of twice the standard error of estimate ( $\pm 10.2$ ). It should be remembered in this connection that in any case the standard deviation of a germination test, where only two replicates of 100 seeds each are used, is high. It is probable that with a larger number of observations and more replications in the germination tests closer agreement would have been obtained.

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# CYCLIC COMPOUNDS CONTAINING A CARBONYL GROUP; A MECHANISM FOR THE FORMATION OF PYRYLLIUM SALTS FROM 1,5-DIKETONES<sup>1</sup>

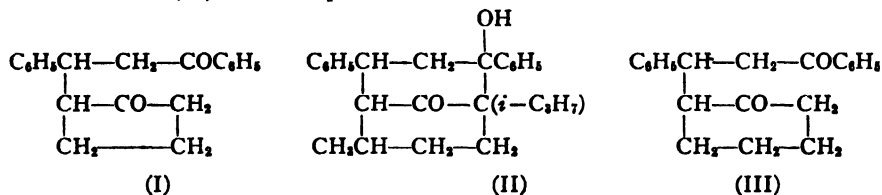
BY C. F. H. ALLEN<sup>2</sup> AND H. R. SALLANS<sup>3</sup>

## Abstract

In the presence of alkali, cyclohexanone and its homologues add to chalcones to form either semicyclic diketones or dicyclic keto-alcohols; the latter contain a carbonyl bridge, and the former can be converted into closed ring structures and dehydrated to form substances having a carbonyl bridge. In these dicyclic ketones the bridge is not removed by heating, in contrast to the behavior of certain other compounds having a similar ring system.

A second mode of ring closure gives rise to pyryllium salts; the isolation of a methyl ether has made it possible to devise a plausible mechanism for this hitherto obscure reaction. Four varieties of salts are described, the perchlorates being obtained by a different procedure than that previously employed.

In continuation of the work on cyclic compounds containing a carbonyl bridge (1, 3), a type first described by Stobbe has been investigated. This author added cyclopentanone (16, 17) and the substituted cyclohexanones, menthone (18) and 3-methylcyclohexanone (16, 17), to benzalacetophenone, obtaining semicyclic compounds like (I), except in the case of menthone, for which two possible bicyclic structures were written. As will be shown later structure (II) is to be preferred.



Some of this work has been repeated and the series extended by the use of other ketones.

Cyclohexanone adds more easily to benzalacetophenone than any of the other cyclic ketones. Since the product forms a dioxime and adds two molecules of Grignard reagent without evolution of gas it is an open-chain 1,5-diketone (III).

The addition product is not noticeably affected by the dehydrating agents used by Stobbe to form bicyclic ketones. Concentrated sulphuric acid readily sulphonates it, but a very small amount of oil can be obtained that on vacuum distillation yields two dehydration products containing but one carbonyl group. By analogy they are the stereoisomeric ketones (IV); only one was obtained in a sufficient quantity to study. It formed a monoxime, and in the Grignard machine added one molecule of methyl magnesium iodide; on

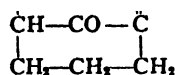
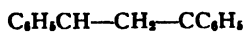
<sup>1</sup> Manuscript received September 5, 1933.

Contribution from the Department of Chemistry, McGill University, Montreal, Canada.

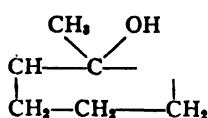
<sup>2</sup> Assistant Professor of Chemistry, McGill University.

<sup>3</sup> Graduate student, McGill University, and during one year holder of a studentship under the National Research Council of Canada.

hydrolysis of the complex a tertiary alcohol (V) was formed. This evolved gas when treated with methyl magnesium iodide and was regenerated on



(IV)

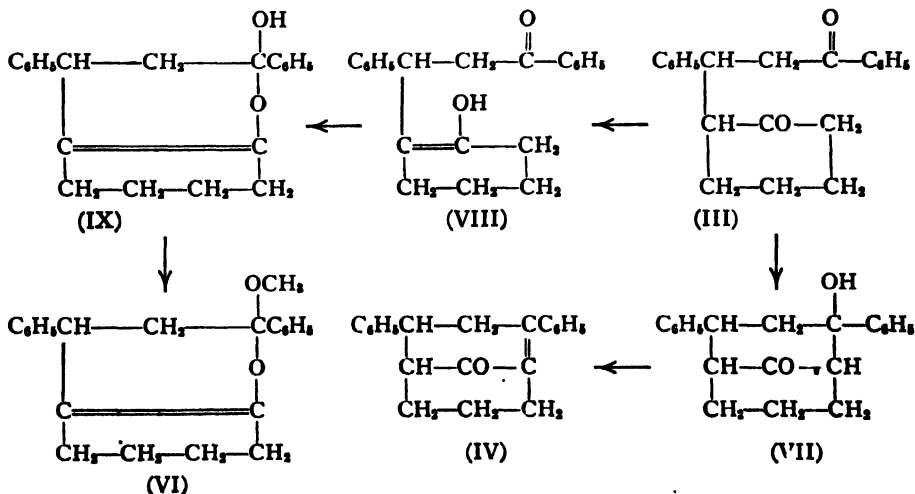


(V)

decomposition of this magnesium complex by acids. Neither one decolorized bromine nor reduced permanganate.

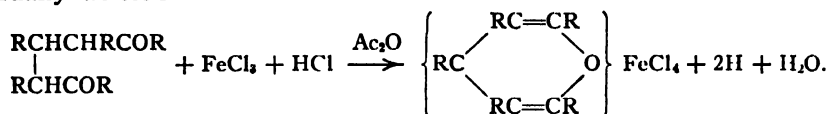
These bicyclic ketones having a carbonyl bridge are stable on heating; they can be distilled *in vacuo* without loss, and there is no evolution of gas when they are heated far above their melting points at ordinary pressure. This is noteworthy in view of the ease with which carbon monoxide is lost when there is an endo CO bridge in a six-membered bicyclic ring (3). Substance (I) would give a derivative of cycloheptane and the others, cyclooctane; from a study of mechanical models it can be seen that both of these types with the bridge are under no strain.

A third substance (m.p. 171° C.) was frequently isolated in small amounts from the action of sulphuric acid on the addition product, as well as by the use of any mineral acid in dry methyl alcohol. It was eventually found that it could be prepared almost quantitatively by the use of alcoholic sulphuric acid. This substance does not give an oxime, semicarbazone or benzoate. In the Grignard machine it neither combines with the reagent nor evolves gas; therefore both carbonyl groups are involved in its formation. By the Zeisel method it shows the presence of a methoxyl group; it does not reduce permanganate nor decolorize bromine. Taking this evidence into account, the substance is best represented as the methyl ether (VI); it is really a hemiacetal formed by methylation of the cyclic isomeric form of the diketone (III). By inspection of the formulas it is seen that cyclicization can occur in two ways:

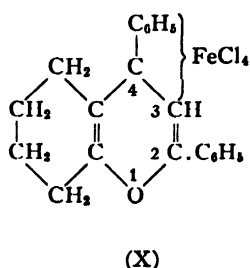
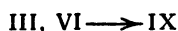


Direct addition of the active hydrogen of the  $\text{CH}_2$  group gives an intermediate of type (VII), like the menthone addition product (II); since this still has an alpha hydrogen it loses water to form the ketones (IV). But if enolization occurs first (VIII) then an alternative mode of addition is possible (IX), and the OH is then acidic enough to form the ether. In agreement with this, the latter is formed from the diketone under conditions used for acetal formation, *e.g.*, by the use of anhydrous copper sulphate in dry methyl alcohol. It is very sensitive to acids, being rapidly hydrolyzed to the diketone.

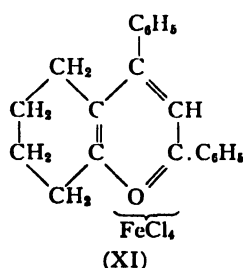
A characteristic reaction of 1,5-diketones is the formation of pyryllium salts on treatment with anhydrous ferric chloride in acetic anhydride. Since the diketones must have a hydrogen in the alpha position to each carbonyl group this suggests the possibility of a connection between cyclicization and enolization. No satisfactory mechanism has yet been proposed—the equation is usually written



Both the addition product (III) and the methyl ether (VI) react with ferric chloride and acetic anhydride to form the same ferrichloride. This suggests that the mechanism is by way of an intermediate cyclic compound like (IX); by loss of water and oxidation of the remaining hydrogen (possibly to OH, followed by salt formation as in the case of the triphenylmethane dyes) the pyryllium compound is formed. The formation of the methyl ether (VI)



or



may be taken as evidence in favor of the first step since it shows that such a bicyclic system is possible; whether dehydration or oxidation occurs next is a matter of uncertainty, but the last step is obviously formation of the salt.

As structures for the salt, either type (X) or (XI) may be considered. The authors prefer (X) for these reasons: (1) in a recent series of papers (8) Diltley has presented very convincing evidence that in a considerable number of pyryllium salts such a system of linkages exists. As an alternative the double bond might be between atoms 3 and 4, and the carbonium atom be

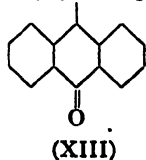
number 2, but it is obviously impossible to locate an electric charge with any degree of certainty. (2) Johnson (9) has examined the absorption spectra of a number of substances having quinoid-like and benzoid-like nuclei, and found that the bands of the pyrones, their acid salts, and N-methyl lutidone are all similar, but radically different from those of completely conjugated systems such as benzene, toluene, and 4-methoxylutidine.

In order to have more than one kind of salt, other reagents have been employed in this work. It was found that the ferrichloride could be easily changed into a crystalline perchlorate; the latter was more conveniently obtained directly from the diketone, the perchloric acid acting as an oxidizing agent. Thus a new method for preparing this type of perchlorates is provided; it was extended to include benzaldiacetophenone and certain homologues, as described in the experimental part. It was also found that stannic and antimonie chlorides could replace ferric chloride, and yield nicely crystalline salts. These are double salts; like ferric chloride, the antimony compound is formed by the addition of one molecule of antimony pentachloride to the chloride of the cyclic derivative, but the tin compound is an addition product of an acetate. Thus the general formulas may be written  $R \cdot FeCl_4$ ,  $R \cdot SbCl_6$ ,  $R(OCOCH_3)SnCl_4$ . The R is the same in each salt, as it is possible to convert one into another.

The diketone and hydroxylamine hydrochloride or the dioxime with hydrogen chloride gives an oily tetrahydroquinoline derivative (XII) that was isolated as a picrate; the formation of substituted pyridines under these conditions is a characteristic property of 1,5-diketones—indeed, it is probable, as Hollins points out (10, p. 213), that when a supposed 1,5-diketone does not give a pyridine it is the isomeric cyclic hydroxy compound. A careful study of a number of these has been made by Rabe (13, 14, 15). In this work the same has been found true of the formation of pyryllium salts.

The structure of the menthone addition product was left undetermined by Stobbe, but since it gave only a monoxime he represented it as a cyclic substance. This has been confirmed by its behavior in the Grignard machine; it consumes two moles of reagent and evolves one mole of gas. Since it cannot be dehydrated without complete decomposition, being recovered unchanged after treatment with all except the most drastic reagents it is best represented as (II). If it had the only possible isomeric structure there would be a hydrogen on the carbon alpha to the carbonyl group (where  $C_3H_7$  now is) and adjacent to the hydroxyl so that water would be easily removable. It does not give a pyryllium salt with ferric chloride and acetic anhydride.

$C_6H_5CHCH_2COC_6H_5$



The formation of a monoxime is not conclusive evidence of the presence of only one carbonyl group in these semi-cyclic compounds. The diketone (XIII) formed by addition of anthrone to benzalacetophenone (12) also gave only a monoxime, but in the Grignard machine it consumed two moles of reagent without evolution of gas.

## Experimental

### A. The Addition Products

The new addition products were all made by the same general method; 4 cc. of 40% sodium hydroxide solution was added to a warm (35–40° C.) mixture of 50 cc. of alcohol, 6 gm. of the cyclic ketone, and 3 gm. of the chalcone—after several hours the crystalline addition product was filtered and washed, first with water and then with ether. It was then recrystallized from an appropriate solvent. An excess of ketone is essential for obtaining the best results. These substances are very readily soluble in hot methyl and ethyl alcohols, acetic acid, chloroform, carbon tetrachloride, and benzene, but sparingly soluble in the cold alcohols, ether, and petroleum ether; the solubility increases with the amount of substitution.

2-Phenacylobenzylcyclohexanone (III) forms white silky needles, m.p. 149° C., when recrystallized by dissolving 18 gm. of the crude product in 25 cc. of hot chloroform and adding 75 cc. of alcohol and 1 cc. of acetic acid; yield, 90–95%. Calcd. for  $C_{21}H_{22}O_2$ : C, 82.4; H, 7.2%; mol. wt., 306. Found: C, 82.1; H, 7.3%; mol. wt., 305. In the Grignard machine it consumed two moles of reagent and showed two additions.

A trimolecular product is formed in the absence of a solvent, using sodium ethylate; it forms fine needles from alcoholic chloroform that melt at 167–168° C. Calcd. for  $C_{36}H_{34}O_3$ : C, 84.0; H, 6.6%; mol. wt., 514. Found: C, 83.7; H, 6.8%; mol. wt., 507. In the Grignard machine it consumed three moles of reagent, showing 2.8 addition and evolving 0.3 mole of gas.

2-(4-Chlorophenacylobenzyl) cyclohexanone (XIV), 2-(4-chlorophenacylobenzyl)-3-methylcyclohexanone (XV), and 2-phenacylobenzyl-3-methylcyclohexanone (XVII) all formed white needles from alcohol.

TABLE I  
PROPERTIES OF CHLOROKETONES

Substance	M.p., ° C.	Yield, %	Formula	% Cl calcd.	% Cl found	Grignard machine
XIV	125–6	90	$C_{21}H_{21}O_2Cl$	10.1	10.2	1.9 addition
XV	155–6	65–70	$C_{23}H_{23}O_2Cl$	10.0	9.9	1.9 addition

The cyclopentanone (I) and menthone (II) addition products were prepared by Stobbe's directions, but the manipulation has to be done with considerable care and oils are nearly always formed. The first was purified by crystallizing from 60–80° C. petroleum ether and the second from 90% alcohol. In the Grignard machine the latter consumed two moles of reagent, evolving one mole of gas and showing one addition. It gave a gum with thionyl and acetyl chlorides, that on hydrolysis regenerated the starting material.

The dioxime of (III), prepared in the usual manner, formed transparent white prisms from ethyl alcohol, m.p. 186° C. Calcd. for  $C_{21}H_{24}O_2N_2$ : 8.3%. Found: N, 8.5.

The pyridine derivative (XII) was made by refluxing for three hours a mixture of 5 gm. of the addition product, an equal weight of hydroxylamine hydrochloride and 100 cc. of alcohol, and pouring the wine-red solution into 300 cc. of water containing 3 gm. of sodium hydroxide. The greenish-yellow gum was removed, dissolved in alcohol and chloroform, and an excess of picric acid added; a picrate separated in shiny, yellow flakes (m.p. 196° C.). Calcd. for  $C_{27}H_{22}N_4O_7$ : N, 10.8%. Found: N, 10.3%. A similar product resulted when the dioxime, suspended in dry benzene, was saturated with hydrogen chloride. Attempts to regenerate a crystalline free base were unsuccessful.

The addition product reacted vigorously with bromine, evolving clouds of hydrogen bromide; the product was a thick gum that contained bromine but could not be recrystallized. It lost part of its bromine to alcoholic potassium acetate, but still remained as a gum.

The anthrone addition product\* (XIII) (12) formed only a monoxime when treated with excess of hydroxylamine, but in the Grignard machine consumed two moles of reagent and showed two additions. The oxime formed fine prisms from methyl alcohol (m.p. 144–145° C.). Calcd. for  $C_{29}H_{23}O_2N$ : N, 3.3%. Found: N, 3.1%.

*B. The Dehydration Products, the Bicyclic Ketones; Bicyclo-[3.1.3]-2,4-diphenyl-9-ketnonene-4, (IV)*

To a solution of 5 gm. of the addition product (III) in 100 cc. of absolute alcohol, concentrated sulphuric acid was added until the solution became permanently red. After 24 hr. the mixture was warmed on the steam bath and water added until the solution became slightly turbid. On cooling 2.6 gm. of a solid separated, that after recrystallization from alcohol formed shiny, transparent plates (m.p. 143° C.). When the orange-red solution of the addition product in cold (10° C.) concentrated sulphuric acid was allowed to stand overnight and poured into ice water most of the product was water-soluble, but the insoluble oil was separated and distilled in a vacuum; 50 gm. of the diketone gave 10 gm. of crude oil that on redistillation yielded 3 gm. of a pale yellow oil (b.p. 155–160° C. at 25 mm.), from which only 0.5 gm. of a crystalline product could be obtained. This was an isomeric dehydration product; it formed prisms when recrystallized from methyl alcohol; m.p. 151° C. Calcd. for  $C_{21}H_{20}O$ : C, 87.5; H, 6.9%. Found: (143° C.) C, 87.4; H, 7.0%; (151° C.) C, 87.4; H, 6.9%. The diketone (III) was recovered unchanged after prolonged refluxing in acetyl chloride or acetic anhydride.

The oxime (from the isomer melting at 143° C.) separated as cubes from 70% alcohol; m.p. 156° C. Calcd. for  $C_{21}H_{21}ON$ : N, 4.6%. Found: 4.4%.

In the Grignard machine, the isomer melting at 143° C. consumed one mole of reagent and showed one addition; 0.2 gm. sample was used, and after making the required measurements, the carbinol formed was recovered by distilling the solvent. After recrystallization from alcohol, 0.05 gm. of pure substance was obtained. Bicyclo-[3.1.3]-2,4-diphenyl-9-methyl-9-hydroxy-

\* The experimental work on this substance, except for the treatment in the Grignard machine, was done by Dr. F. B. Wells.



nonene-4 (V) formed square-ended plates, m.p. 147° C. Calcd. for  $C_{22}H_{24}O$ : C, 86.8; H, 7.9%. Found: C, 86.8; H, 8.0%. It evolved gas when treated with methyl magnesium iodide, but was recovered after acidification with no change in melting point or mixed melting point.

*C. The Methyl Ether; 2,4-Diphenyl-2-methoxyheptahydrobenzopyrane (VI)*

Although obtained in a variety of ways, the best procedure was as follows: 10 gm. of the addition product (III) was dissolved in 50–60 cc. of chloroform, an equal volume of absolute methyl alcohol and 1 cc. of constant-boiling hydrobromic acid added, and the solution allowed to evaporate spontaneously. The residual solid was recrystallized, the analytical sample from methyl alcohol. It formed needles; m.p. 171° C.: yield, 7 gm. Calcd. for  $C_{22}H_{24}O_2$ : C, 82.5; H, 7.5;  $OCH_3$ , 9.7%. Found: C, 82.1; H, 7.3;  $OCH_3$ , 9.9%. It was also obtained from the gum formed by the action of sulphuric acid on the addition product, or on allowing a methyl alcoholic solution containing any mineral acid or anhydrous copper sulphate to stand for some time. The methyl ether is very slightly soluble in methyl and ethyl alcohols, but dissolves readily in hot *n*-propyl and *n*-butyl alcohols, amyl ether, ethyl acetate, acetic acid or anhydride, chloroform and benzene. There was no reaction of any kind with the Grignard reagent, hydroxylamine, nor permanganate. It did not decolorize bromine instantly, but copiously evolved hydrogen bromide like the diketone (III) and formed a gum. On warming a solution in ordinary alcohol containing a trace of sulphuric acid it was rapidly hydrolyzed to the original addition product. It gave the pyryllium salts described below.

None of the other addition products, nor several other previously known 1,5-diketones gave a methyl ether—its isolation in this instance was doubtless fortuitous, and possible only because of its insolubility.

*D. The Pyryllium Salts*

Four varieties of these were prepared as stated in the introduction. Those containing a metal were made by the usual procedure for ferrichlorides, but the perchlorates were made more easily as follows. To a suspension of 2 gm. of the diketone in 8 cc. of acetic anhydride and an equal volume of absolute ether, 1.5 cc. of 50% perchloric acid was added, drop by drop—if too rapidly, the mixture became hot or even exploded. In all instances the perchlorates separated as needles on standing—the yields were of the order of 50% for diketones like benzaldiacetophenone, but only 20% in the case of the semicyclic substances. They were recrystallized from glacial acetic acid containing a drop of perchloric acid. The metal salts were recrystallized from a 1:1 mixture of acetic acid and anhydride. The antimony salts did not separate readily from the gummy by-products and could not be isolated at all from the semicyclic diketones.

*Example of interconversion of salts.* The tin salt (2 gm.) was suspended in 5 cc. of acetic anhydride, and 2 gm. of ferric chloride or 1.5 cc. of perchloric acid added; the mixture became hot. On cooling, 1.2–1.5 gm. of the ferrichloride or perchlorate separated, analytically pure.

In addition, to the new addition products and the methyl ether described above, benzaldiacetophenone (XVIII) (11) and a phenylated homologue (XIX) (2, formula VI) were used. The results are summarized in the tables.

TABLE II  
FERRICHLORIDES;  $\text{RFeCl}_4^*$

Diketone	Formula	M.p., °C.	Crystal form	% Fe calcd.	% Fe found
III	$\text{C}_{21}\text{H}_{19}\text{OFeCl}_4$	161	Yellow needles	11.5	11.6
XVII	$\text{C}_{22}\text{H}_{21}\text{OFeCl}_4$	133	Yellow prisms	11.2	11.4
I	$\text{C}_{20}\text{H}_{17}\text{OFeCl}_4$	126	Brown prisms	11.8	11.8

\* Cf. Ref. 2 for others related to this work.

TABLE III  
PERCHLORATES;  $\text{RCIO}_4$

Diketone	Formula	M.p., °C.	Color	% Cl calcd.	% Cl found†
III	$\text{C}_{21}\text{H}_{19}\text{O}_2\text{Cl}$	214	Yellow	9.1	9.2
XVII	$\text{C}_{22}\text{H}_{21}\text{O}_2\text{Cl}$	231	Orange-yellow	8.8	8.9
I	$\text{C}_{20}\text{H}_{17}\text{O}_2\text{Cl}$	240 d.	Yellow	9.5	9.4
XVIII†	$\text{C}_{22}\text{H}_{21}\text{O}_2\text{Cl}$	—	Brown-yellow	—	—
XIX	$\text{C}_{20}\text{H}_{19}\text{O}_2\text{Cl}$	258	Yellow	7.3	7.3

† The chlorine was determined by the method of Willard and Thompson (19); direct titration was unsatisfactory.

‡ Previously made in another way by Dilleky (6).

TABLE IV  
TIN SALTS;  $\text{R} \cdot \text{C}_2\text{H}_5\text{O}_2 \cdot \text{SnCl}_4$

Diketone	Formula	M.p., °C.	Crystal form	Analyses		
					Calcd.	Found
III	$\text{C}_{22}\text{H}_{22}\text{O}_2\text{SnCl}_4$	143	Yellow-orange prisms	Cl Sn †† $\text{C}_2\text{H}_5\text{O}_2$ ††	23.4 19.6 9.7	23.0 19.4 10.0
XVII	$\text{C}_{24}\text{H}_{24}\text{O}_2\text{SnCl}_4$	135	Yellow prisms	Cl Sn	22.9 19.2	22.6 19.0
I	$\text{C}_{21}\text{H}_{20}\text{O}_2\text{SnCl}_4$	161	Brown prisms	Cl Sn	23.9 20.5	23.6 20.3
XVIII	$\text{C}_{22}\text{H}_{20}\text{O}_2\text{SnCl}_4$	205	Lemon-yellow needles	Cl Sn $\text{C}_2\text{H}_5\text{O}_2$	22.6 18.9 9.4	22.3 18.6 9.7
XIX	$\text{C}_{21}\text{H}_{18}\text{O}_2\text{SnCl}_4$	206	Orange needles	Cl Sn	20.4 17.1	20.1 17.2

†† Estimated as  $\text{SnO}_2$ .

‡‡ Perkin's acetyl method (4, p. 284).

TABLE V  
ANTIMONY SALTS; RSbCl<sub>6</sub>

Diketone	Formula	M.p., °C.	Crystals	% Cl calcd.	% Cl found
XVIII	C <sub>22</sub> H <sub>17</sub> OSbCl <sub>6</sub>	320 d.	Brown prisms	33.1	32.9
XIX	C <sub>29</sub> H <sub>21</sub> OSbCl <sub>6</sub>	233 d.	Brown prisms	29.5	29.1

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## PYROLYSIS OF THE LOWER PARAFFINS

### III. PRODUCTION OF OLEFINS IN BAFFLED METAL TUBES<sup>1</sup>

BY ADRIEN CAMBRON<sup>2</sup> AND COLIN H. BAYLEY<sup>3</sup>

#### Abstract

Results have been obtained which indicate that the conversion of the lower paraffins to olefines by thermal treatment can be satisfactorily carried out in special alloy steel tubes at 800–820° C. By using baffled tubes it has been found possible to obtain high rates of conversion at temperatures considerably lower than when using open tubes. Actually the temperature has been brought within the range of usefulness of special alloy steels. Heat-resistant alloys of the 18–8 type have been found unsuitable for this purpose, because nickel appears to catalyze the formation of elementary carbon, but nickel-free alloys containing over 20% of chromium have been found satisfactory.

The production of olefines by the thermal dehydrogenation of the lower paraffin hydrocarbons in metal tubes has been investigated by Chamberlin and Bloom (3). These authors, however, were mainly concerned with the production of aromatics, which are produced when the thermal treatment is carried out under such conditions that the olefines formed as primary products are allowed to polymerize. They investigated tubes of steel, nickel, Monel metal and copper, none of which were suitable, inasmuch as they catalyzed the formation of carbon from the gas, or else underwent failure due to carburization at the high temperature necessary for the reaction.

More recently Podbielniak (7) has described results obtained on a semi-commercial plant scale both on the thermal dehydrogenation of the lower paraffins to olefines and on the polymerization of the olefines to aromatics. This author employed special alloy steel tubes arranged in a single coil and externally heated in a gas-fired furnace. Waste stabilizer gases from petroleum refining were used as raw material. By careful regulation of the conditions of temperature and flow it was possible to obtain good yields of olefines and aromatics. However, there was always a more or less considerable deposition of carbon. This could be partly overcome by increasing the velocity of passage of the gas and by careful temperature control, but it was nevertheless necessary to "blow" the tubes with steam every 8 to 12 hr. in order to remove the carbon deposit.

The economic possibilities of the production of olefines by thermal treatment of natural gas fractions or waste refinery gases have been reviewed by several authors (1, 5, 7). Olefines are now being, or have been, produced commercially by the thermal treatment of natural gas fractions, but no data are available regarding the yield of olefines and it is not known whether conditions have been found under which the deposition of carbon is avoided.

<sup>1</sup> Manuscript received November 1, 1933.

Contribution from the National Research Laboratories, Ottawa, Canada.

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<sup>3</sup> Junior Research Chemist, National Research Laboratories, Ottawa.

In a recent publication from these laboratories (2) are given the results of experiments on the thermal dehydrogenation of the lower paraffins to olefines in quartz tubes, under conditions of turbulent flow resulting from the use of specially designed baffles. It is shown in that paper that, other factors being constant, the yield of olefines is considerably higher under conditions of turbulent flow than when the gas flow is streamline. It is also shown that, for a given rate of flow of gas or vapor, the temperature required for a given amount of decomposition to olefines is considerably lower when the gas flow is turbulent. It was also observed that, owing to the more uniform heating of the gas under conditions of turbulent flow, it is possible to obtain a product containing much higher concentrations of olefines without loss of the olefines through side reactions, such as polymerization or breakdown to carbon and hydrogen.

Owing to the high cost and fragility of quartz, porcelain and other refractory tubes and also to constructional difficulties, the practicability of using these materials in commercial scale operation is doubtful. It was consequently thought advisable to investigate the suitability of heat-resistant alloy steel tubes, especially since it had been found possible, by carrying out the reaction under conditions of turbulent flow, to bring the reaction temperature within the range where such tubes can be used.

Experiments have been carried out with two types of alloys. The first alloy investigated was of the KA2S type containing approximately 18% of chromium and 8% of nickel. The second alloy contained 28% of chromium and only traces of nickel.

### Apparatus

The apparatus used to measure and control the gas flow and to remove any liquids from the gaseous products was the same as previously described (2). The furnace was built in three sections, two of which are shown in Fig. 1. The lengths of the sections were 32, 58 and 58 cm. respectively, the heating of each section being independently controlled. The first and third sections were wound in one circuit, whilst the second section was wound in two circuits. Thus the complete furnace consisted of four independently controllable sections. The distance between the windings and the wall of the reaction tube was 2.2 cm.

The tube was supported axially by means of perforated plates attached to the ends of the furnace. Openings through the furnace insulation permitted thermocouples to be introduced into the space between the windings and the reaction tube.

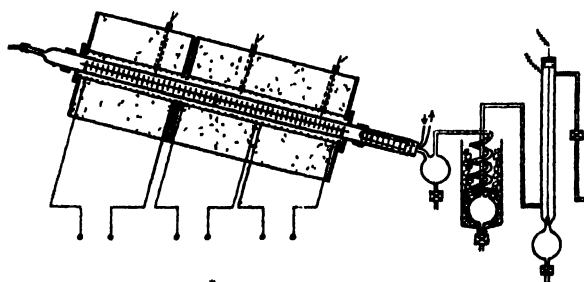


FIG. 1. *Diagram of apparatus.*

## Gases Used

The propane and *n*-butane were supplied in cylinders by the Carbide and Carbon Chemicals Corporation. The values of *n* in  $C_{2n+2}$  for each paraffin, determined by slow combustion analysis, are as follows:—propane, 2.95; *n*-butane, 3.98.

## Experimental

## (A) Experiments in KA2S Tubes

(i) *Pyrolysis of propane in 58-cm. furnace.* Experiments were carried out in an alloy steel tube of the KA2S type (chromium : nickel, 18 : 8). The diameter of the tube was 2.7 cm., the heated length being 58 cm. In one series of experiments, mica baffles of 2.4 cm. diameter were used, mounted on an 0.8-cm. quartz rod and spaced 1.5 cm. apart. A second series of experiments was carried out without baffles. The results are given in Table I. These show the very pronounced increase in the yield of olefines at a given temperature when the gas flow is rendered turbulent by means of suitably designed baffles. It will be observed, for instance, that in experiments Nos. 79 and 82 the yield of olefines was increased from 259 to 359 gm. per hour when the gas flow was rendered turbulent.

TABLE I  
PYROLYSIS OF PROPANE IN BAFFLED AND IN EMPTY KA2S TUBES

Expt. No.	Rate, l./hr.	Ex- pansion, %	Temp., °C.	Analysis of gaseous products, % by vol.				Olefines pro- duced, gm./hr.	Carbon, gm./hr.
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	H <sub>2</sub>		
Baffled tube									
79	416	69.4	923	1.2	25.0	10.5	17.2	359	—
80	411	64.2	912	0.7	23.8	11.6	16.5	369	0.5
81	414	81.2	949	1.3	26.7	9.6	18.2	386	—
Empty tube									
82	406	50.6	925	0.5	19.6	9.5	14.5	259	—
83	413	59.1	954	0.7	21.0	9.7	15.0	292	1.5
84	411	66.9	977	1.1	21.4	9.8	17.1	297	—

(ii) *Pyrolysis of n-butane in 58-cm. furnace.* Experiments were also carried out with *n*-butane using the same furnace as in the previous propane experiments, with baffles. The results are given in Table II.

TABLE II  
PYROLYSIS OF *n*-BUTANE IN BAFFLED KA2S TUBE

Expt. No.	Rate, l./hr.	Ex-pansion, %	Temp., °C.	Analysis of gaseous products, % by volume					Ole-fines, gm./hr.	% Conversion by weight entering <i>n</i> -butane
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	C <sub>4</sub> H <sub>6</sub>	H <sub>2</sub>		
85	422	74.9	886	0.5	16.4	19.0	1.5	11.1	443	40.6
86	410	94.9	904	0.9	19.5	19.4	0.5	11.7	484	45.7

Even at such high conversions as in experiment No. 86, for instance, where 45.7% by weight of the entering *n*-butane was converted into olefines, very little of the olefines are lost through side reactions. Considering the small reaction volume employed, it is also worthy of note that over one pound of olefines is produced per hour.

(iii) *Pyrolysis of propane in 85-cm. furnace.* In order further to reduce the temperature required for the conversion of propanes to olefines, the furnace length was increased. The *KA2S* tube was used and all of these experiments with the exception of No. 90 were carried out with baffles, the latter consisting of circular disks 2.3 cm. in diameter, cut from *KA2S* sheet 0.75 mm. thick, supported 1.5 cm. apart on a 0.65-cm. rod of the same material, the baffles being placed throughout the heated length.

These experiments were carried out particularly to determine the effect of increasing the length of the furnace on the temperature required for a given amount of decomposition, but also to determine the effect of turbulence at the lower temperature and to make a quantitative estimate of the amount of carbon formed in tubes of this alloy. The results obtained in these experiments are recorded in Table III.

TABLE III  
PYROLYSIS OF PROPANE IN *KA2S* TUBE, 85-CM. FURNACE

Expt. No.	Temp., °C.	Gas rate, l./hr.	Ex-pansion, %	Analysis of gaseous products, % by volume				Olefines produced		Carbon formed, gm./hr.
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	H <sub>2</sub>	Gm./hr.	% yield by wt.	
87	850	376.5	80.2	1.0	28.2	10.5	18.4	372.7	50.5	9.6
88	850	375	81.3	—	—	—	—	—	—	8.8
89	850	378	79.0	1.2	28.3	10.5	18.6	373.7	50.3	5.4
90	850	384	54.2	0.7	21.9	10.4	16.2	278.0	36.9	5.0
91	860	608	75.2	0.4	25.5	12.4	17.9	588.0	48.5	1.7
92	860	582	74.0	0.5	26.5	13.1	18.3	559.5	49.2	1.7
93	860	573	71.7	0.6	25.9	12.2	18.0	553.7	49.3	9.6
94*	860	573	38.5	0.8	18.5	8.9	20.2	317.0	28.3	5.1
95*	860	582	40.2	0.4	17.4	8.9	—	318.3	28.0	10.8

\* No baffles used.

A comparison of experiments Nos. 87 and 89 with No. 81 shows that the amounts of olefines produced per hour are substantially the same at 850° C. in the 85-cm. furnace as at 949° C. in the 58-cm. furnace.

Although the temperature used in these experiments was lower than those in the preceding experiments (Table I), the observed effect of turbulence on the reaction was just as marked. The amounts of olefines produced in experiments Nos. 87 and 89, for instance, were of the order of 373 gm. per hour whilst in experiment No. 90, without baffles, the yield of olefines was only 278 gm. per hour. Carrying out the reaction under conditions of turbulent flow, therefore, increased the yield of olefines by 35%. At a higher gas rate

the effect of turbulence is considerably more marked. Thus in experiments Nos. 92 and 93 the yield of olefines was of the order of 555 gm. per hour, when using the baffled tube, whilst in experiments Nos. 94 and 95 under the same conditions of gas rate and temperature, the yield of olefines was only 318 gm. per hour, when using the empty tube. The use of baffles, consequently, caused a 75% increase in the yield of olefines at the higher gas rate.

Considerable variations were observed in carbon formation under apparently identical conditions. It was expected that the metal would lose its activity on prolonged use. This effect, however, did not appear to take place. Extraction of the carbon with dilute hydrochloric acid indicated the presence of appreciable amounts of iron and nickel. There is some doubt as to whether the nickel is alone responsible for catalyzing the formation of carbon, later work having shown that iron as well as nickel may, under certain conditions, cause the deposition of carbon in the reaction tube.

It will be observed that the amounts of carbon formed in the above experiments are considerably higher than in the experiments given in Table I. This may be due to the fact that mica baffles were used in the latter experiments, whilst metal baffles were used in the former.

#### (B) *Experiments in Nickel-free Chromium Alloy Steel Tubes*

In the following experiments, heat-resistant alloy tubes containing about 28% of chromium and only a trace of nickel were used. The tubes were 2.54 cm. in internal diameter. The baffles, which were used in all experiments except No. 96, were 2.25 cm. in diameter and placed 1.7 cm. apart, the baffles and the 0.65-cm. rod used as support being of the same composition as the tube. The results of the experiments are shown in Table IV in which the first three experiments were carried out in an 85-cm. furnace, and the last two in a 140-cm. furnace.

TABLE IV  
PYROLYSIS OF PROPANE IN 28% CHROMIUM ALLOY TUBE

Expt. No.	Temp., °C.	Gas rate, l./hr.	Ex-pansion, %	Analysis of gaseous products, % by volume				Olefines produced	
				C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub>	Ole-fines, gm./hr.	Yield by weight, %
96*	860	518	59.8	2.2	29.1	8.8	15.5	438	42.3
97*	860	557	84.0	1.2	27.1	11.4	17.6	568	50.2
98†	842	393	79.3	0.6	25.1	14.1	17.5	407.5	53.2
99†	800	411	78.5	1.2	25.3	11.7	20.6	392	48.8
100†	820	517	80.1	1.3	27.2	10.7	16.6	508	50.4

\* *Propane-butane-ethane mixture* ( $n=3.06$ ). † *Propane* ( $n=2.95$ ).

Comparison of experiments Nos. 81, 98 and 99 in which substantially the same gas rate was used, shows that the increased time of contact due to the increased length of the furnace produces a very marked lowering in the temperature required for the same amount of conversion. At 400 litres per hour, the same amount of reaction takes place in the 140-cm. furnace at 800° C. as in the 58-cm. furnace at 949° C.



Experiments Nos. 96 and 97 show the effect of the baffles on the yield obtained when a mixture of propane and *n*-butane containing a little ethane ( $n=3.06$ ) is pyrolyzed at 860° C. in an open and baffled tube. It will be seen that the use of baffles raises the yield of olefines by approximately 30%.

The amount of carbon depositing in the reaction tube or on the baffles was found to be negligible under the above conditions. When the high-chromium alloy steel tube was first used, it was found that a thin very adherent film of hard, grey carbon deposited on the surface of the metal. This film is very similar to that which deposits on an inert surface such as quartz under the same conditions. Its rate of growth on the high-chromium alloy was determined and found to be of the order of 0.007 mm. in 11 hr. This includes the rapid growth when the tube is first used. Examined under the microscope, the film appears to consist of a conglomerate of small spheres fused together, the surface being highly reflecting.

High-chromium steel tubes, as will be reported in a later communication, have also been used in the investigation of the conversion of propane to aromatics at temperatures of 800–850° C. Although the tendency to form carbon is greater under these conditions, the only carbon deposited in the tube is the exceedingly thin film referred to above.

### (C) Recycling Experiments in Nickel-free Chromium Alloy Steel Tubes

These experiments were made in an attempt to increase the time of contact of the gas undergoing pyrolysis so as to reduce the temperature required, by recycling a portion of the exit gas through the furnace. It was found that, even under conditions such that a portion of the olefines underwent polymerization, the percentage conversion to olefines remained substantially constant.

In these experiments the liquids formed were removed from the exit gas by activated charcoal. The 85 by 2.54 cm. furnace was used in experiments Nos. 101–104 and the 140 by 2.54 cm. furnace in experiments Nos. 105–107, the baffles being as in (B) above. The results are given in Table V.

TABLE V  
RECYCLING EXPERIMENTS IN NICKEL-FREE CHROMIUM ALLOY STEEL TUBE

Expt. No.	Temp., ° C.	Gas rate, l./hr.	Re-cycling rate, l./hr.	Ex-pansion, %	Analysis of gaseous products, % by volume				Conversion to liquids based on entering paraffin, % by weight	Olefines produced	
					C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub>		Yield, gm./hr.	Yield, % of paraffin passed
101*	820	227	224	84	0.9	30.5	7.5	16.4	Not determined	220	48.3
102	835	208	228	94	0.0	27.9	6.0	18.8	9.1	186.5	45.8
103	840	211	237	98.5	1.0	27.6	8.0	19.5	10.0	211.3	50.3
104	850	212	230	98.5	1.7	29.0	3.6	22.2	11.5	180.4	43.5
105	810	210	220	95.5	0.7	24.8	3.4	18.3	9.6	220	53.5
106	830	210	234	97.5	0.7	24.0	4.3	20.2	14.1	167.9	40.9
107	850	204	238	106	1.4	24.7	1.7	22.2	20.2	141.1	35.4

\* Propane-butane-ethane mixture. All other experiments with propane.

It will be observed that the combined yields of olefines and liquids expressed as percentage by weight of paraffin put through are quite constant at 55–60% except in experiment No. 105 made at a much lower temperature. It will also be noted that whilst the combined yield of olefines and liquids remains constant, the yields of liquids vary from 9.1 to 20.2% by weight and the yields of olefines from 35.4 to 53.5%. This would seem to show that, under the above conditions, the paraffin loss through methane formation takes place predominantly in the cracking stage, and not in the polymerization stage, for the amount of olefines which disappears when the temperature is raised can be almost totally accounted for by the increased yield of liquids.

### Behavior of Heat-resistant Alloy Steels at Pyrolysis Temperatures

The high-chromium alloys have a considerably lower tensile strength at temperatures above 800° C. than the *KA2S* alloys. For this reason it was found desirable to keep the temperature as low as possible in the experiments with alloys of the former type. For instance, the high-chromium alloy used has a tensile strength of 18,000 lb. per sq. in. at 800° C. and this drops to 8,500 lb. per sq. in. at 900° C. whilst a 20 : 10 chromium-nickel alloy has a tensile strength of 50,700 lb. at 800° C. and 30,000 lb. per sq. in. at 900° C. Consequently, tubes of the high-chromium alloy would show a greater tendency to undergo deformation at elevated temperatures due to plastic flow or "creep". The creep rates, that is, the stress required to produce an elongation of  $10^{-7}$  per hour, for instance, bear about the same ratio to each other as do the tensile strengths of the two alloys (6). A stress of 0.45 tons per sq. in. produces a creep rate of  $10^{-7}$  per hour in a 27% chromium alloy at 650° C., while 2.46 tons are required to produce the same creep rate in an 18–8 chromium-nickel alloy. No deformation has been observed in the 18–8 alloy tubes used in the present experiments, even after 40 to 50 hr. use at temperatures as high as 930° C. With the 28% chromium alloy tube, however, a perceptible sagging of the tube was observed after about  $3\frac{1}{2}$  hr. at 840° C., but tubes of the same alloy were used for several hours at 800° C. without any noticeable deformation. The unsupported length of tube in the above experiments was 85 cm. It is obvious that by providing an adequate number of supports, so as to decrease the unsupported length of tube in the furnace, any difficulty due to sagging could be eliminated. Using a 20% chromium alloy instead of the 28% alloy used in the present experiment would also help in overcoming difficulties due to sagging of the tubes, for the former alloy shows an appreciably lower creep rate at 800–850° C. than the latter.

With regard to the probable life of 28% chromium alloy steel tubes under continuous use, no visible signs of failure are shown by tubes which have been in use for 55–60 hr. at temperatures between 800 and 850° C. In fact, it is reported (4, 8) that tubes of this alloy have been in successful use for over two years in vapor-phase cracking units operating at 870° C.

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## PYROLYSIS OF THE LOWER PARAFFINS

### IV. THE CONVERSION OF GASEOUS PARAFFINS TO AROMATIC HYDROCARBONS IN BAFFLED QUARTZ TUBES<sup>1</sup>

BY ADRIEN CAMBRON<sup>2</sup> AND COLIN H. BAYLEY<sup>3</sup>

#### Abstract

The polymerization of gaseous olefine mixtures, such as are obtained by the pyrolysis of the lower paraffins, has been studied by passing the gases through heated quartz tubes under conditions of streamline and of turbulent gas flow. It is shown that the observed rate of polymerization of the gaseous olefines to liquid hydrocarbons is appreciably higher, at a given wall temperature, under conditions of turbulent flow. By subjecting propane to two thermal treatments at a maximum temperature of 950° C., 29% by weight of the gas is converted to liquids of which 70% boil in the gasoline range, and over 30% by weight of the propane is recoverable in the gaseous by-products as olefines, the total conversion into useful products being consequently about 60%. Substantially the same results have been obtained in single-stage recycling experiments.

The production of aromatic hydrocarbons by the thermal treatment of the lower paraffin hydrocarbons, above methane, has been the subject of numerous investigations. The work carried out up to 1916 has been reviewed by Lomax *et al* (11). More recently, Egloff (7) and his coworkers have published a review covering the work done on the subject up to 1930. The results of some recent investigations have been reported by Frey and Hepp (8), Pease (12), Frolich (9) and Groll (10).

The possible utilization of the gaseous paraffins for the production of aromatics or liquid fuels has been discussed by Bowen and Nash (1) and by Dunstan (5, 6). Dunstan states that on account of the necessity for high temperatures and the need of operating at ordinary pressure resulting in low throughput, the commercial possibilities of such a process have hitherto been regarded as doubtful. It would seem, however, that both of these difficulties can be largely overcome by the use of specially designed reaction tubes in which a high degree of turbulence is induced in the gas flow through the reaction chamber, the considerable increase in the rate of heat transfer from tube wall to gas under these conditions resulting in high rates of conversion even at relatively low temperatures.

The work on the pyrolysis of the lower paraffins, carried out in these laboratories so far, has been concerned with the conversion of the lower paraffins to olefines (2, 3, 4), particularly with the object of determining the effect of turbulence on the reaction. This work has shown that at a given temperature and at high rates of flow, the observed rate of decomposition of the gaseous paraffins into olefines is markedly increased by artificially inducing turbulence in the gas flow through the reaction space.

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The conversion of paraffin to liquid hydrocarbons takes place in two stages; (i) the breakdown of the paraffins into olefines, and (ii) the polymerization of the olefines to liquids. The present investigation has been undertaken in order to determine the effect of turbulence on the rate of polymerization of olefines to liquids. It was not expected that the effect of turbulence would be as marked in the second stage as in the first, since the latter involves endothermic reactions and consequently the amount of reaction is more dependent on the rate of heat transfer from tube wall to gas than the second stage which involves exothermic reactions. The results show, however, that the effects of turbulence upon the second stage are also very marked, the observed rate of polymerization being not only higher than is the case with streamline flow at the same wall temperature, but also that the more uniform temperature distribution throughout the reaction chamber, under conditions of turbulence, suppresses the formation of excessive amounts of tar whilst the formation of carbon is practically negligible.

### Materials Used

The propane and *n*-butane used in these experiments were obtained from the Carbide and Carbon Chemicals Corporation. The values of *n* in  $C_nH_{n+2}$ , as determined by slow combustion analysis were 2.95 and 3.98 respectively.

### Apparatus

The apparatus used in these experiments is shown in Fig. 1. The gas pressure was reduced to about 20 lb. by means of a reducing valve and the gas flow regulated through a low pressure valve *B*, whence the gas passed to the capillary flowmeter *B*<sub>1</sub>, the wetmeter *C*<sub>1</sub>, the drying tower *D* containing fused calcium chloride and to the furnace *F*. The quartz rod carrying the mica baffles was supported usually at three points by triangles of heavy mica sheet mounted on the rod and fitting the tube snugly. The gas leaving the

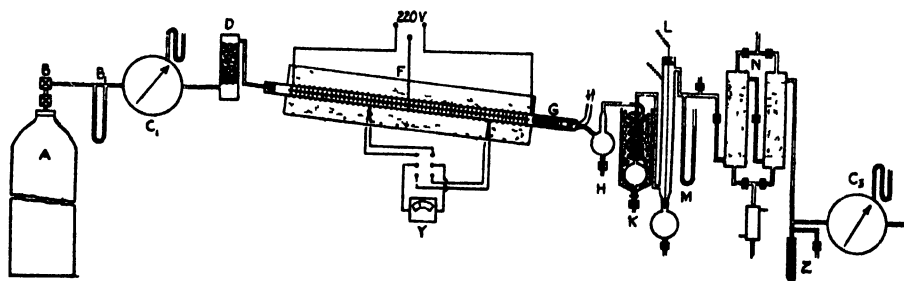


FIG. 1. Furnace used in single-cycle pyrolysis.

furnace was rapidly cooled by means of a loop of  $\frac{3}{8}$ -in. copper tubing to which a number of copper baffles *G* were soldered and through which cold water was passed. The gas then passed through a receiver *H*, a spiral glass condenser *K* immersed in cold water, and an electrostatic precipitator *L*. The recovery of the low-boiling liquids present in the gas was effected, in the

earlier experiments, by passing the gas leaving the precipitator through three tetralin washing towers. At the end of the experiment the low-boiling liquids were removed from the tetralin by distillation up to  $190^{\circ}\text{C}$ . through an efficient column. In the later experiments, the tetralin washers were replaced by charcoal absorbers *N*. The rate of the scrubbed gas from the tetralin or charcoal absorbers was measured by the wetmeter *C*, care being taken to allow the absorbers to become saturated with the gas before taking a reading. Samples of the gas were taken for analysis at *Z*.

Fig. 2 is a photograph of the four charcoal absorbers used. The gas to be treated was passed through the valve *A*, entering the absorber at the base, the treated gas passing out at *B*. At the end of the experiment the valves *A* and *B* were closed, the valves *C* and *D* opened and the charcoal steamed out at a temperature of  $300^{\circ}\text{C}$ . To accomplish this, water was allowed to drop from the flask *E* into the vaporizer *F*, whence the steam passed to the superheater *G*, which was kept at a temperature of  $500^{\circ}\text{C}$ . and then to the absorber through valve *C*. To reduce the amount of steam required, the absorbers were electrically heated by means of nichrome windings and well insulated. The four absorbers were so arranged that they could be used singly or in series. The vapors and steam from the charcoal were passed through the copper coil condenser *H* and collected. The light-oil layer was separated, dried over fused calcium chloride and weighed.

The method used in the analysis of the gaseous products was as previously given (3).

The tar which collected in the condensers and in the electrostatic precipitator was distilled and the fraction boiling below  $200^{\circ}\text{C}$ . was added to the low-boiling liquids recovered from the tetralin or charcoal absorbers. In general the liquids removed from the exit gases by the tetralin or charcoal absorbers consisted mainly of benzene with a little toluene whilst most of the toluene and practically all the xylenes and higher aromatics were present in the tar from the condenser and precipitator. In these experiments, the fraction boiling above  $200^{\circ}\text{C}$ . is referred to as "tar" and the fraction boiling below  $200^{\circ}\text{C}$ . as "light oil".

As the result of later work in metal tubes, an account of which is to be published shortly, sufficiently large amounts of light oil and tar were pro-



FIG. 2. Charcoal absorbers.

duced, by the pyrolysis of propane, to permit of the determination of the composition of these liquids by distillation and chemical methods.

### Experimental

#### (a) *Pyrolysis of Propane in 80-cm. Furnace (Single-stage Experiments)*

The following series of experiments was carried out using a quartz reaction tube, the heated length being 80 cm. and the diameter 2.5 cm. The tube was electrically heated by means of nichrome resistance ribbon wound directly on it and well insulated against heat losses. The heated length of the furnace was divided into two equal sections, the current supplied to each being controlled separately. Thermocouples were placed in contact with the outside wall of the reaction tube at a point two-thirds the distance along each of the heated sections.

Experiments 1-3 were carried out to determine the effect of varying the gas rate on the yield of liquids, whilst the object of Experiments 4-11 was to determine the effect of turbulence on the yield and on the relative amounts of light oil and tar formed under conditions of turbulent and streamline flow at different temperatures, the inlet rate being held as constant as possible.

Experiments 4-7 were carried out with baffles of 2.1-cm. diameter spaced 1.8 cm. apart in the second section, whilst in Experiments 8-11 no baffles were used in this section. In order to determine the effect of turbulence on the polymerization stage of the conversion of propane to liquids, the heating of the first, or cracking section was controlled so as to obtain as complete cracking as possible before the gas entered the second, or polymerization section. In all experiments baffles were used in the first section. Reference to Experiment 38, Table XV, in a previous communication (3, p. 189), shows that at the temperature and gas rate used, the gas entering the second section contains approximately 25% of ethylene and 11% of propylene.

TABLE I

PYROLYSIS OF PROPANE TO LIQUIDS IN 80 BY 2.5 CM. FURNACE

Expt. No.	Temp., 0° C.		Rate, l/hr.	Ex-pansion, %	A	B	C	D	E	F	G
	1*	2*									
1	913	900	245	119	45.3	56.5	35.0	9.4	11.5	8.9	3.4
2	930	930	342	101	54.2	54.7	41.7	8.1	9.9	7.6	3.3
3	964	965	384	102	91.7	40.1	69.2	12.2	14.9	11.3	3.1
4	958	950	364	105.5	80.8	55.3	62.9	12.0	13.8	10.7	3.5
5	957	972	378	99.0	94.6	51.8	71.3	13.5	15.5	11.9	3.1
6	958	990	368	106	110.8	49.4	75.3	16.3	18.7	12.7	2.1
7	959	1010	357	118	121.9	45.1	68.4	18.4	21.2	12.0	1.3
8	958	951	364	86	53.6	52.7	42.7	8.0	9.2	7.2	3.6
9	958	975	372	92	67.2	53.8	48.7	9.8	11.3	8.2	2.6
10	958	1005	374	93.2	84.1	49.5	57.0	12.1	13.9	9.4	2.1
11	957	1046	379	109	108.4	48.4	66.5	15.5	17.8	11.0	1.6

\* Temperatures of first and second furnace sections respectively.

In this series of experiments the low-boiling liquids were removed from the exit gas by washing with tetralin.

The results obtained are shown in Table I. In this and subsequent tables, the letters used as column headings refer to the following data:—*A*, total yield of liquids in grams per hour; *B*, percentage by weight of the propane put through recoverable as olefines from the exit gas; *C*, yield of light oil, grams per hour; *D*, percentage yield of liquids based on weight of propane put through; *E*, yield of liquids in lb. per 1,000 cu. ft. propane put through; *F*, yield of light oil lb. per 1,000 cu. ft. propane put through; *G*, ratio light oil : tar.

The results of the analyses of the gaseous products obtained in the above experiments are shown in Table II.

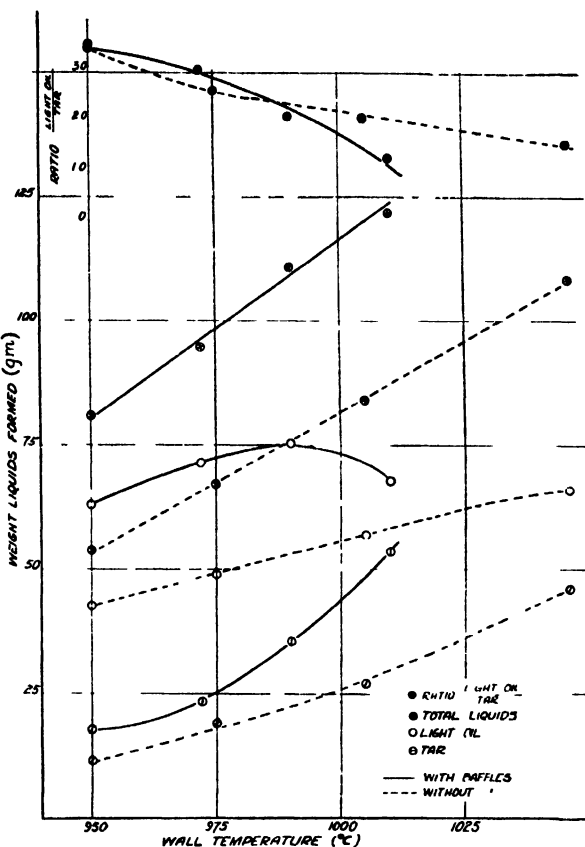


FIG. 3. Effect of baffles on the yield and character of the liquids produced.

TABLE II  
ANALYSIS OF GASEOUS PRODUCTS, EXPERIMENTS NOS. 1-11

Expt. No.	% by volume					<i>n</i> for residue
	C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub>	Residue	
1	1.9	29.6	5.7	20.8	42.0	1.11
2	1.8	30.0	6.8	20.5	40.9	1.03
3	3.7	27.4	2.4	23.8	42.7	1.07
4	2.7	30.8	4.8	22.1	39.6	1.09
5	2.7	28.1	5.2	23.5	40.5	1.03
6	3.5	26.9	3.3	25.5	40.8	1.03
7	4.4	24.1	1.7	29.6	40.2	1.02
8	1.6	28.5	8.0	19.7	42.2	1.22
9	2.1	29.0	6.4	20.9	41.6	1.17
10	2.2	27.6	5.6	22.7	41.9	1.15
11	2.5	25.9	4.1	25.4	42.1	1.10



It will be observed that at 950° C. a 50% increase in the yield of liquids is obtained by carrying out the polymerization stage of the reaction under conditions of turbulent flow. Similar results are obtained at higher temperatures.

The variations with temperature, in the yields of total liquids, light oil and tar, under conditions of streamline and turbulent flow are shown in Fig. 3.

Comparison of Experiment 4 (with baffles) and Experiment 10 (without baffles), shows that at a given rate of flow, 12% of the propane is converted to liquids when the polymerization stage is carried out at 950° C. under conditions of turbulent flow, whilst a temperature of 1005° C. is required in order to obtain the same percentage conversion under conditions of streamline flow. Under the latter conditions the light-oil-tar ratio of the product is considerably lower.

It should be pointed out that the yields of liquids in the second series of experiments would have been considerably lower if no baffles had been used in the cracking section of the furnace, because the effect of turbulence on the cracking stage is very much more marked than on the polymerization stage.

#### (b) Two-stage Pyrolysis Experiments

In the following series of experiments the gas was subjected to two thermal treatments, the liquids formed in the first stage being removed before the gas entered the second furnace. This was done primarily to determine whether the temperature at which a reasonably high yield of liquids are obtained could be brought down to the range in which special alloy steel tubes could be used.

The length of the first furnace was 80 cm. and of the second, 100 cm.; the diameters of both furnaces being 2.5 cm. Baffles of 2.1-cm. diameter were used throughout the length of both sections. After each thermal treatment the gas was passed through a condenser and Cottrell precipitator and the liquids remaining in the gas were recovered by absorption in three tetralin washing towers connected in series. The rates of flow of the tetralin through the towers were so adjusted that the tetralin leaving the last tower was free from absorbed liquids. The results are shown in Table III.

TABLE III  
TWO-STAGE PYROLYSIS OF PROPANE TO LIQUIDS IN 180 BY 2.5 CM. FURNACE

Expt. No.	Temp., ° C.					Rate, l/hr.	Expansion, %	A	B	C	D	E	F	G
	1*	2*	3†	4†	5†									
12	920	912	888	812	762	253	113	109.7	31.6	71.3	22.2	27.1	17.7	1.9
13	920	908	815	850	846	248	88.2	104.4	36.1	78.7	21.5	26.4	19.9	3.1
14	922	920	893	865	815	248	117	147.0	30.6	72.5	30.3	37.0	18.2	0.96
15	927	918	850	850	850	245	110	119.5	32.9	83.6	24.8	30.4	21.3	2.3
16	920	898	800	820	840	244	97.5	80.3	44.3	64.0	17.8	20.4	16.3	4.0
17	925	905	850	850	850	244	97.5	104.3	36.9	74.7	23.1	26.6	19.1	2.5

\* Temperatures of sections of first furnace. † Temperatures of sections of second furnace.

The results of the analyses of the off-gas after the second thermal treatment are given in Table IV. In Experiment 17 an analysis was carried out on the gas leaving the first stage.

TABLE IV  
ANALYSIS OF GASEOUS PRODUCTS, EXPERIMENTS NOS. 12-17

Expt. No.	% by volume					n for residue
	C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>3</sub> H <sub>6</sub>	H <sub>2</sub>	Residue	
12	2.3	20.5	1.9	28.2	47.1	1.09
13	1.5	25.2	2.1	24.0	47.2	1.02
14	2.6	19.8	1.6	30.0	46.0	1.07
15	1.6	22.6	1.4	25.4	49.0	1.01
16	1.6	27.3	3.0	22.1	46.0	1.00
17a*	2.0	30.0	4.8	21.7	41.5	1.22
17b†	2.1	23.5	1.5	26.0	46.9	1.05

\* Analysis of gas after first stage.

† Analysis of gas after second stage

The maximum conversion to liquids obtained in the above experiments was 30.3% by weight of the propane put through (Experiment 14). This represents a yield of 37 lb. per 1,000 cu. ft. of gas used, the light-oil-tar ratio being 0.96. Although the total conversion to liquids was only 24.8% in Experiment 15, the conversion to light oil was higher than in the previous experiment, the yield of liquids boiling in the gasoline range being 21.3 lb. per 1,000 cu. ft. The density of the light oil being 0.83, this corresponds to a yield of 2.57 gal. of light oil per 1,000 cu. ft. of propane put through. Since the amount of olefines in the off-gas represents a conversion to ethylene and propylene of 32.9% of the propane put through, the total conversion to aromatics and olefines is 57.7%. These results compare very favorably with those reported by Podbielniak (13) who, using a gas of substantially the same composition as the gas used in the present experiments, obtained a yield of light oil of 1.8 gal. per 1,000 cu. ft. of gas passed.

### (c) Effect of Temperature and Time of Contact on Light-oil-tar Ratio

The following experiments were carried out to determine the effect of raising the temperature and lowering the time of contact on the light-oil-tar ratio of the liquids formed in the second furnace. In Experiments 18-22, 23-26 and in Experiment 27, the length of the second furnace was 120 cm., 90 cm. and 60 cm. respectively. The diameter of the furnace was 2.5 cm. in all cases.

In order to recover the liquids present in the gas after each thermal treatment, the gas was first cooled to 15-20° C., passed to the electrostatic precipitator to remove the tar fog and finally through a charcoal absorber containing 400 gm. of activated charcoal. (Although such a procedure would undoubtedly be found less economical than absorption by means of oil or tetralin on a commercial scale, the use of charcoal for recovering the liquids formed by

pyrolysis has been found much more convenient in the laboratory.) The results obtained are given in Table V.

TABLE V  
TWO-STAGE PYROLYSIS OF PROPANE TO LIQUIDS

Expt. No	Temp., ° C.					Rate, l./hr.	Expansion, %	A	B	C	D	E	F	G
	1*	2*	3†	4†	5†									
18	955	970	903	900	890	382	108	157.4	29.9	106.2	21.1	25.8	17.5	2.1
19	950	950	930	930	910	363	111	207.5	29.9	140.4	29.3	35.8	24.2	2.1
20	953	965	902	923	922	375	128	197.9	28.2	135.6	27.0	33.0	22.6	2.2
21	952	956	935	935	935	358	120	202.7	27.1	135.1	29.0	35.4	23.6	1.7
22	952	970	945	950	950	383	110	235.8	19.3	141.2	31.5	38.4	23.1	1.5
23	952	970	—	956	953	373	107	175.2	29.6	119.4	24.1	29.4	20.0	2.1
24	950	956	—	960	960	360	116	178.4	27.9	116.9	25.4	31.0	20.3	1.9
25	960	962	—	970	970	375	114	211.3	25.5	129.2	28.8	35.1	21.6	1.6
26	955	958	—	1003	998	383	123	213.0	—	150.3	28.5	34.7	24.5	2.5
27	952	958	—	—	1027	360	112	140.1	36.5	99.0	20.0	24.4	17.3	2.2

\* Temperatures of sections of first furnace. † Temperatures of sections of second furnace.

In all the above experiments, with the exception of No. 18, the amounts of liquids formed during each thermal treatment were recovered separately. This was done in order to determine the relative light-oil-tar ratio of the products obtained in each stage. The results are shown in Table VI.

TABLE VI  
LIGHT-OIL-TAR RATIOS OF LIQUIDS FORMED IN TWO FURNACES

Expt. No.	Liquids formed in first furnace			Liquids formed in second furnace		
	Total liquids, gm.	Light oil, gm.	Light-oil-tar ratio	Total liquids, gm.	Light oil, gm.	Light-oil-tar ratio
19	98.2	75.2	3.3	115.1	68.6	1.5
20	98.0	76.1	3.5	99.9	59.5	1.5
21	81.2	59.5	2.7	121.5	75.6	1.6
22	104.9	80.9	3.4	130.9	60.3	0.85
23	104.9	76.2	2.6	70.3	43.2	1.6
24	75.1	54.8	2.7	103.3	62.1	1.5
25	113.5	79.0	2.3	97.8	50.2	1.1
26	119.6	91.3	3.3	93.4	59.0	1.75
27	88.8	64.5	2.7	51.3	34.5	2.1

The results of the analyses of the off-gas from these experiments are shown in Table VII. In several experiments, a sample of the gas was taken after the first thermal treatment in order to determine the composition of the gas entering the second furnace.

TABLE VII

ANALYSIS OF GASEOUS PRODUCTS, EXPERIMENTS 18-27

Expt. No.	% by volume					n for residue
	C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	H <sub>2</sub>	Residue	
18	2.4	19.2	2.1	30.1	46.2	1.06
19A	3.3	27.9	5.0	22.6	41.2	1.00
19B	2.9	19.5	1.6	27.9	48.1	1.04
20	2.9	19.1	0.0	32.3	45.7	1.00
21A	3.4	28.0	5.2	22.4	41.0	1.01
21B	3.1	17.2	1.6	30.7	47.4	1.00
22	3.0	11.8	1.9	37.7	45.6	1.01
23	3.2	20.0	1.4	30.6	44.8	1.00
24A	3.1	28.5	4.8	22.4	41.2	1.06
24B	3.2	18.7	0.8	31.8	45.5	1.02
25	3.5	16.2	1.5	31.9	46.9	1.00
26*	—	—	—	—	—	—
27A	3.0	28.2	5.2	22.4	41.2	1.02
27B	3.3	20.4	4.1	27.7	44.5	1.01

\* No gas sample.

A, gas sample taken after first furnace.

B, gas sample taken after second furnace.

The results of these experiments show that raising the temperature, and decreasing the time of contact so that the amount of total liquids formed remains constant, has no marked effect on the light-oil-tar ratio. This would seem to show that the relative rates of formation of light oil and tar are not, under these conditions, appreciably affected by temperature. Thus, for instance in Experiment 20, the total yield of liquids from the second furnace was 99.9 gm. when the heated length was 120 cm. and the temperature 920° C., the light-oil-tar ratio being 1.5. In Experiment 24, in which the heated length of the second furnace was reduced to 90 cm. and the temperature increased to 960° C., the total quantity of liquids formed was 103.3 gm., the light-oil-tar ratio being also 1.5.

When the length of the second furnace was reduced to 60 cm. (Expt. 27), the temperature could not be raised sufficiently high to obtain the same conversion as in the preceding experiments. The high light-oil-tar ratio of the liquids obtained in Experiment 27 was due to the smaller amount of liquids formed, as compared with the preceding experiments.

It will also be observed that when the conditions were such that the amounts of liquids formed in the first furnace were equal to, or greater than, the amounts of liquids formed in the second furnace, the light-oil-tar ratio of the liquids from the first furnace were consistently higher than those from the second furnace. This is believed to be due to the fact that the concentration of the olefines in the gas entering the second, or polymerization, section of the first furnace was considerably higher than that in the gas entering the second furnace. Since the rate of polymerization of the olefines, other

conditions being equal, increases with their concentration, it follows that a shorter time of contact is required at a higher concentration of olefines to produce a given amount of liquids which, being subjected to a high temperature for a shorter time, would be less likely to undergo side reactions.

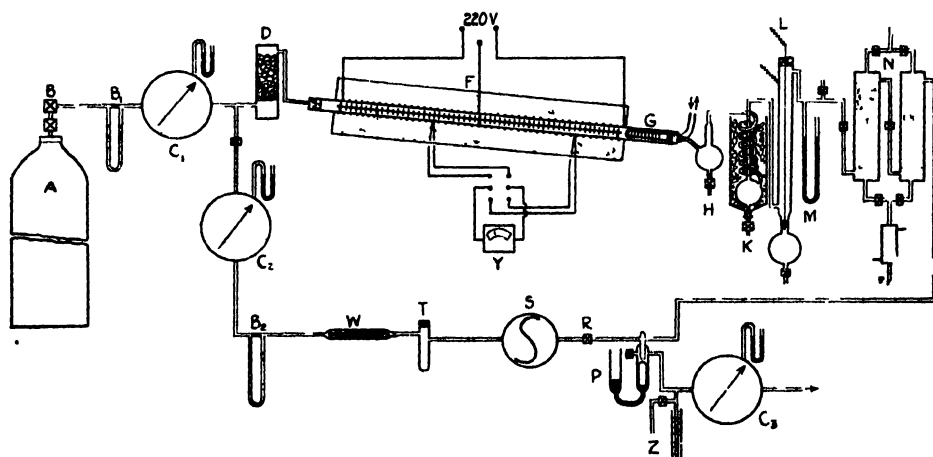


FIG. 4. Furnace used in recycle experiments.

#### (d) Recycling Experiments

The following series of experiments was carried out in order to compare the yields of liquids obtained when the gas is given two consecutive thermal treatments, as in (b), with the yields obtained when a fraction of the off-gas is recycled through the furnace. Fig. 4 is a diagram of the apparatus used.

A fraction of the scrubbed gas leaving the charcoal absorbers was again put through the reaction tube by means of the control valve *P*, the Milvay pump *S*, the oil trap *T* and the oil-spray trap *W*, and mixed with fresh gas before entering the furnace. It was found preferable to use castor oil in the

TABLE VIII  
RECYCLING EXPERIMENTS

Expt. No.	Temp., ° C.		Rate, L./hr.		Expansion, %	A	B	C	D	E	F	G
	1*	2*	†	††								
28	950	960	77.3	425	127	33.9	25.0	24.0	22.9	27.9	19.7	2.4
29	950	955	95.0	371	136	59.7	—	39.0	32.2	39.2	25.5	1.9
30	932	940	114	334	128	45.1	36.6	20.7	25.2	17.5	12.2	2.3
31	953	953	119	361	130	51.7	—	35.4	22.2	27.0	18.5	2.2
32	960	955	118	376	130	63.8	—	45.2	27.7	33.7	23.9	2.4
33	943	942	142	259	113	66.1	—	45.0	23.8	29.0	19.7	2.1
34	930	935	80.3	234	91	38.3	—	25.5	25.0	30.4	20.3	2.0
35	923	922	113	376	152	71.6	34.1	52.0	24.8	39.7	28.8	2.65
36	952	955	113	389	156	87.7	21.2	59.9	30.3	48.5	33.0	2.15

\* Temperatures of first and second section of furnace.

† Inlet rate.

†† Recycling rate.

pump rather than a hydrocarbon oil owing to the lower solubility of hydrocarbon gases in the former. A wet meter  $C_2$  and flowmeter  $B_2$  placed between the pump and the reaction tube served to measure the volume of gas recycled. The remainder of the gas passed from the exit side of the valve  $P$  to the wet meter  $C_3$  and thence to the hood. Samples of exit gas were taken for analysis at  $Z$ .

Table VIII shows the results obtained with different temperatures, inlet rates and recycling rates. Propane was used in Experiments 28–34, and *n*-butane in Experiments 35 and 36.

The results of the analyses of the gaseous products obtained in four of the above experiments are shown in Table IX.

TABLE IX  
ANALYSIS OF GASEOUS PRODUCTS

Expt. No.	% by volume						$n$ for residue
	$C_2H_2$	$C_2H_4$	$C_2H_6$	$C_4H_8$	$H_2$	Residue	
28	2.6	16.8	0.2	—	30.4	50.0	1.00
30	1.7	21.5	2.2	—	26.4	48.2	1.00
35	1.9	23.0	3.2	0.0	21.4	50.5	1.04
36	2.4	14.9	1.4	0.0	17.1	64.2	—

It will be observed that the best yields obtained under the conditions of these experiments are about the same as in the experiments in which the gas was subjected to two thermal treatments; also that the capacity of a given size of reaction tube, *i.e.*, the amount of liquids produced per hour, is about the same in both cases. It would consequently appear that the use of recycling is more practical than subjecting the gas to two thermal treatments, since in the latter case two separate liquid recovery systems including condensers, electrostatic precipitators and absorbers must be used whilst in the former case only one liquid recovery system is required.

The light-oil-tar ratio of the liquids obtained in the recycling experiments was, on the whole, appreciably higher than the liquids obtained by two thermal treatments.

The results obtained in these experiments show that, by increasing the length of the furnace, the temperature required for the conversion of the gaseous paraffins to liquids can be brought within the range of usefulness of heat-resisting alloy steels.

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# STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

## IV. THE DECOMPOSITION OF PARA-*n*-BUTYRALDEHYDE AND PARA-ISOBUTYRALDEHYDE<sup>1</sup>

BY C. C. COFFIN<sup>2</sup>

### Abstract

The gaseous decompositions of para-*n*-butyraldehyde and para-isobutyraldehyde to *n*-butyraldehyde and isobutyraldehyde respectively are homogeneous and first order over the pressure and temperature range investigated (1.3 to 55 cm. of mercury; 215 to 261° C.). Under these conditions the reactions go to completion at a measurable rate without complications. Within experimental error the activation energies of these reactions are equal and are approximately the same as that of the paracetaldehyde decomposition. This value is between 42,000 and 44,000 calories per mole. The rates of decomposition of the two parabutyrals are very nearly the same at any temperature. At 500° abs. the velocity constant of the iso-compound is about 15% greater than that of the normal and about 100% greater than that of paracetaldehyde. The velocity constants at any temperature are given by the

equations: para-*n*-butyraldehyde,  $\ln k = 33.12 - \frac{42,000}{RT}$ ; para-isobutyralde-

hyde,  $\ln k = 34.06 - \frac{42,800}{RT}$ . The data are consistent with the idea that, for a series of reactions with the same energy of activation, an increase in the number of contributory internal degrees of freedom of a molecule will increase the probability of reaction.

### Introduction

A study of the decomposition kinetics of various organic compounds in the gaseous state is being made in this laboratory (1, 2, 3, 4.). The present paper is concerned with the thermal breakdown of para-*n*-butyraldehyde and para-isobutyraldehyde to *n*-butyraldehyde and isobutyraldehyde respectively. As might be expected, these decompositions are very similar and closely resemble that of paracetaldehyde (4). Over the pressure and temperature range (1.3 to 55 cm. Hg; 215 to 261° C.) so far investigated all three reactions are homogeneous and first order. The pressure increase in each case is quite accurately 300%, so that the decompositions are for practical purposes complete. Within the probable limit of experimental error the activation energies of the three reactions are the same; *viz.*, 42,000 to 44,000 calories per mole. In accordance with the idea that increase of molecular complexity at constant temperature should increase the reaction probability of an activated molecule if the activation energy remains unchanged (3), the parabutyrals have been found to react somewhat faster than paracetaldehyde over the temperature range investigated. The two isomers have almost identical velocity constants at any temperature—those of the iso-compound being slightly the greater.

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## Experimental

### *Apparatus and Technique*

The low volatility of the parabutyrals necessitated some changes in the apparatus previously described (4). The vapor jacket on the manometer tube leading to the reaction chamber was replaced by a close-fitting heavy-walled copper tube wound with nichrome wire and heavily lagged with asbestos. The manometer could thus be heated electrically to any desired temperature. Small windows on opposite sides of the copper tube enabled the mercury surface to be brought to a definite level. A view of the mercury surface was necessary also to observe the time of explosion of the small bulb containing the reactant and to make sure that all of the reactant evaporated. A mercury thermometer, the bulb of which was sunk in the wall of the copper tube, served to indicate the temperature which was regulated by hand, with suitable rheostats and kept constant to within  $0.5^{\circ}\text{C}$ . during a run. The readings of this thermometer were plotted against the pressures registered by the manometer when the mercury was held in position with the reaction chamber evacuated. The curve obtained was used in correcting the observed pressures of the velocity measurements. As this correction, which is the resultant of the vapor pressure of the mercury and the difference in density of the hot and cold sides of the manometer, is purely empirical, a knowledge of the true temperature of the mercury surface is not necessary in obtaining the actual pressure of the reaction mixture. A voltmeter across the heater was found convenient in correcting for changes in line voltage before they could affect the temperature.

The first 38 runs with para-*n*-butyraldehyde were made in the apparatus just described. At this point an explosion (due probably to a leak of air through a crack in the glass) destroyed this set-up, which was replaced by a larger one consisting of a one-litre round-bottomed Pyrex flask (reaction chamber) sealed inside a similar flask of two-litre capacity (the mercury vapor jacket). The manometer below the reaction chamber was heated electrically as already described. The mercury was introduced by hydrostatic pressure through a three-way tap and all-glass connections from an overhead reservoir. It was removed through the other way of the stopcock to a trap which could be emptied by a tap at the bottom. This mercury was cleaned with nitric acid and distilled before being returned to the reservoir so that only clean mercury which had not come in contact with rubber could enter the apparatus. A 50-cc. Pyrex Erlenmeyer flask, to the bottom of which finely broken glass was fused to prevent bumping (6), served as the mercury boiler. It was heated electrically. A supplementary heater (coils of nichrome wire embedded in the heavy lagging of asbestos cement about the two-litre flask) was necessary at the higher temperatures. The mercury boiler was connected to a 20-litre volume, the pressure of which could be kept constant to well within 0.1 mm. by an automatic regulator described elsewhere (5).

This apparatus has proved to be very satisfactory. It may be left to itself for an indefinite time—frequently running for weeks without a shut-

down. It is customary to allow the reaction to finish during the night (the temperature is raised temporarily toward the end if necessary) and the final pressure which is needed in the calculations is determined the next day before starting another run. At the higher temperature several runs may be made in one day.

In order to diminish the effect of any systematic errors the runs were made in as random an order as was conveniently possible. The experiments listed in Table I were interspersed with many velocity measurements on various other paraldehydes and esters.

#### *Purification of the Paraldehydes*

The first 38 runs with para-*n*-butyraldehyde and the first 10 runs (also the bulb experiments to be described) with the iso-compound were made with samples distilled from material supplied by the Eastman Kodak Company. The remaining runs in each case were made with a very small middle fraction obtained after several fractional distillations (*n*-compound) and sublimations (iso-compound) *in vacuo* of a relatively large quantity of the original material. A special all-glass apparatus without stopcocks was used in each case. As no difference in reaction velocity before and after purification was apparent in either compound it was probable that no catalytic impurities were present. It may be mentioned here that the erratic behavior occasionally observed in the paracetaldehyde decompositions (4) was less noticeable with para-*n*-butyraldehyde and was practically absent in the case of para-isobutyraldehyde. As the decompositions of the latter compound are the most reproducible of the three reactions, the data obtained in this case are considered to be the most reliable.

#### *Reaction Products*

As no products other than the aldehydes themselves appear possible from such low temperature depolymerizations of the para compounds, no attempt was made to identify the substances produced in the decompositions. The fact that the pressure just triples and that the observed and calculated final pressures agree as closely as can be measured, is considered to be sufficient proof that the decompositions are not complicated by side reactions. The products in each case are quite stable at the highest temperatures employed, the final pressures increasing only very slightly over a period of several days.

### Results

#### *Velocity Constants*

As in the case of paracetaldehyde (4) the expression  $\log \frac{2 P_0}{3 P_0 - P}$  was plotted against time (seconds) and a velocity constant determined for each run from the slope of the best straight line through the points,  $\{k \text{ (sec}^{-1}) = \text{slope} \times 2.3\}$ . In no case did these points deviate appreciably from a straight line before the reaction was about 75% complete (*i.e.*,  $\log \frac{2 P_0}{3 P_0 - P} = 0.6$ ;  $P = 2.5 P_0$ ; mole fraction of para compound remaining = 0.1). A small drift up or down after this point was occasionally observed, particularly in the case of the

runs at the lower pressures, and in all probability is simply due to the increasing importance of the error in  $P_0$  as  $3P_0 - P$  diminishes. As already mentioned the para-*n*-butyraldehyde runs resemble those of paracetaldehyde in occasionally showing a greater deviation from the mean than the probable experimental errors will account for, *e.g.*, runs Nos. 25 to 29, Table I. These irregularities, which have not yet been explained nor eliminated, are most apparent at pressures below about 5 cm. and may be due to traces of catalysts.

The results obtained with the apparatus described above are given in Table I, the headings of which are in most cases self-explanatory. In column No. 3 are listed the approximate pressures of mercury vapor in the reaction chamber. It will be observed that these pressures vary from 1.2 to over 80 mm. of mercury without appreciably affecting the reaction velocities. The observed initial pressures (column No. 4) are actually one-third of the observed final pressures. It is impossible to determine the initial pressures directly with sufficient accuracy. The pressures of column No. 5 were calculated by the ideal gas laws from the weight of material taken. In general the agreement between the observed and calculated values is so good that it is immaterial which is used for evaluating  $k$ . In columns Nos. 7 and 8 are given the mean values of  $1/T$  and  $-\log k$  for all the runs at any one temperature. Runs Nos. 25 to 29 with para-*n*-butyraldehyde were omitted in calculating  $-\log k$  for 527.1° abs. as the velocity constants of these runs seem suspiciously high. No explanation can be offered for this discrepancy although the presence of air was suspected. Accordingly runs Nos. 30 and 31 were made with about 2 mm. air pressure in the reaction chamber and runs Nos. 32, 33, 34 and 35 were started in an exceptionally high vacuum ( $<10^{-4}$  cm. Hg). As Table I shows, a few millimetres pressure of air has no appreciable catalytic effect.

TABLE I  
SUMMARY OF RESULTS

Run No.	Temp., ° abs.	$P_{Hg}$ , mm.	$P_0$ (obs.), cm. Hg	$P_0$ (calcd.), cm. Hg	$k$	$\frac{1}{T} \times 10^4$	$-\log k$
Para- <i>n</i> -butyraldehyde							
7	494.5	17	—	28.65	$6.8 \times 10^{-4}$	2.022	4.17
16	494.5	2.4	—	5.50	$6.7 \times 10^{-4}$		
38	494.5	33	—	54.90	$6.6 \times 10^{-4}$		
51	494.5	12	—	10.35	$6.9 \times 10^{-4}$		
39	501.9	17	9.42	9.64	$1.2 \times 10^{-4}$	1.992	3.92
40	502.0	12	2.49	2.35	$1.2 \times 10^{-4}$		
42	501.9	12	4.00	4.11	$1.2 \times 10^{-4}$		
3	510.8	17	—	24.25	$2.6 \times 10^{-4}$	1.957	3.58
4	510.8	18	22.62	22.64	$2.6 \times 10^{-4}$		
5	510.8	17	4.46	4.12	$2.5 \times 10^{-4}$		
6	510.8	16	4.71	4.29	$2.5 \times 10^{-4}$		
11	510.8	53	17.42	17.45	$2.6 \times 10^{-4}$		
12	510.8	53	1.78	1.82	$2.7 \times 10^{-4}$		
13	510.8	13	3.34	3.33	$2.6 \times 10^{-4}$		
14	510.8	2.4	2.34	2.38	$2.6 \times 10^{-4}$		
15	510.8	2.4	2.25	2.30	$2.7 \times 10^{-4}$		

TABLE I—*Concluded*

SUMMARY OF RESULTS

Run No.	Temp., ° abs.	$P_{\text{Hg.}}$ , mm.	$P_o$ (obs.), cm. Hg	$P_o$ (calcd.), cm. Hg	$k$	$\frac{1}{T} \times 10^4$	$-\log k$
Para- <i>n</i> -butyraldehyde— <i>Concluded</i>							
43	522.0	12	6.13	6.47	$6.0 \times 10^{-4}$	1.915	3.22
44	522.0	12	3.25	3.25	$6.3 \times 10^{-4}$		
45	522.0	2.2	1.37	1.36	$6.0 \times 10^{-4}$		
46	522.0	17	12.00	12.12	$6.0 \times 10^{-4}$		
47	522.0	14	—	3.64	$5.6 \times 10^{-4}$		
1	527.2	9	12.73	—	$9.6 \times 10^{-4}$	1.897	3.01
2	527.2	9	10.67	—	$9.6 \times 10^{-4}$		
9	527.1	15	14.75	14.93	$9.7 \times 10^{-4}$		
10	527.1	7	3.18	3.21	$9.4 \times 10^{-4}$		
19	527.1	15	7.79	8.47	$9.0 \times 10^{-4}$		
20	527.1	15	8.11	8.60	$9.0 \times 10^{-4}$		
21	527.1	17	3.34	3.43	$9.2 \times 10^{-4}$		
22	527.1	17	2.16	2.42	$8.7 \times 10^{-4}$		
23	527.1	17	13.79	14.63	$9.2 \times 10^{-4}$		
24	527.1	2.4	5.43	5.68	$10.3 \times 10^{-4}$		
25	527.1	2.4	4.38	—	$11.5 \times 10^{-4}$		
26	527.1	1.2	1.92	—	$11.2 \times 10^{-4}$		
27	527.1	1.8	2.13	—	$11.5 \times 10^{-4}$		
28	527.1	2.4	1.65	—	$12.0 \times 10^{-4}$		
29	527.1	1.5	1.34	—	$14.6 \times 10^{-4}$		
30	527.1	1.5	1.28	—	$9.6 \times 10^{-4}$		
31	527.1	1.5	4.04	—	$10.7 \times 10^{-4}$		
32	527.1	1.5	2.11	—	$10.0 \times 10^{-4}$		
33	527.1	1.5	3.48	—	$9.6 \times 10^{-4}$		
34	527.1	*82	25.20	—	$9.4 \times 10^{-4}$		
35	527.1	82	3.93	—	$10.6 \times 10^{-4}$		
36	527.1	1.2	1.93	—	$11.0 \times 10^{-4}$		
37	527.1	82	25.04	26.32	$9.3 \times 10^{-4}$		
48	527.0	17	20.0	20.9	$9.6 \times 10^{-4}$		
49	527.0	33	2.5	2.1	$10.0 \times 10^{-4}$		
50	527.0	17	24.3	25.3	$9.7 \times 10^{-4}$		
Para-isobutyraldehyde							
3	488.2	2.4	7.30	7.21	$4.4 \times 10^{-3}$	2.048	4.36
10	488.0	2.6	6.15	6.17	$4.2 \times 10^{-3}$		
11	493.5	3.6	16.45	16.49	$6.9 \times 10^{-3}$	2.027	4.16
12	493.5	3.0	11.80	11.89	$6.8 \times 10^{-3}$		
13	506.2	2.6	9.04	8.99	$2.1 \times 10^{-3}$	1.975	3.68
14	506.2	2.2	7.88	7.87	$2.1 \times 10^{-3}$		
1	515.0	3.9	8.48	8.17	$4.2 \times 10^{-3}$	1.942	3.38
4	515.0	1.3	3.52	3.35	$4.0 \times 10^{-3}$		
5	515.0	1.3	1.87	1.82	$4.4 \times 10^{-3}$		
2	527.0	2.8	9.60	9.57	$1.2 \times 10^{-3}$	1.898	2.95
6	526.7	1.3	1.91	1.85	$1.0 \times 10^{-3}$		
15	526.7	1.6	5.59	5.73	$1.1 \times 10^{-3}$		
16	526.8	2.4	8.82	9.19	$1.1 \times 10^{-3}$		
17	527.0	2.8	10.74	11.02	$1.1 \times 10^{-3}$		
18	527.0	2.2	6.75	6.97	$1.1 \times 10^{-3}$		
19	526.8	3.0	11.63	12.04	$1.2 \times 10^{-3}$		
7	534.2	3.9	—	10.81	$1.7 \times 10^{-3}$	1.872	2.74
9	534.2	2.8	9.60	9.25	$1.9 \times 10^{-3}$		

### Homogeneity of the Reaction

With the object of testing the homogeneity of the reactions and of obtaining by an entirely different method a further check on the data in Table I, a few experiments were carried out in sealed glass tubes. These tubes (made of thin-walled Pyrex 2–3 cm. inner diameter; 30 to 100 cc. capacity) were provided at one end with a capillary tube ending in a small (1–2 cc.) bulb into which the contents of the tube could be frozen, sealed off, and finally analyzed by a melting point determination. The surface-volume ratio could be increased by the addition of lengths of glass tubing. Small glass capsules containing a weighed amount of para-isobutyraldehyde (the reaction mixtures

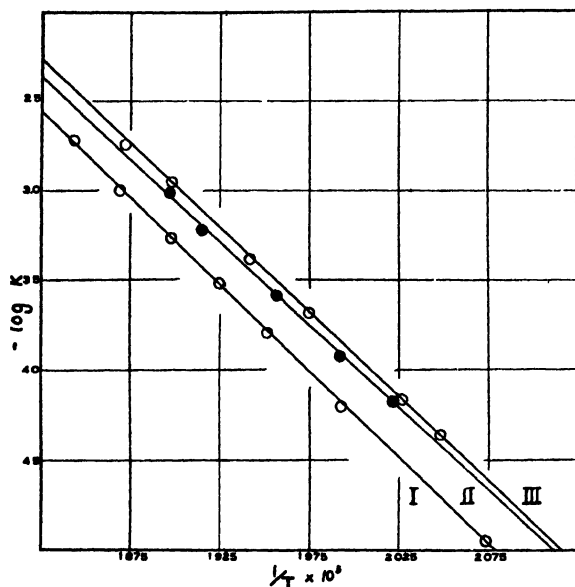


FIG. 1.  $-\log k$  plotted against  $\frac{1}{T}$ . Curve I. Paracetaldehyde. Curve II. Para-n-butyraldehyde. Curve III. Para-isobutyraldehyde.

so that an approximate analysis of the material in the small bulb was afforded by a melting point determination. The volumes of the tubes were ascertained after the reaction and the initial pressures were calculated from the weight of material taken.

Twelve such tubes were run at 506.5° abs. for 150 min. The initial pressures varied from 54 to 183 cm. of mercury and the surface-volume ratio from 2.3 to 15.4 cm<sup>-1</sup>. (The surface-volume ratio of the one-litre reaction chamber is about 0.5 cm<sup>-1</sup>). All the melting points were between 6 and 10° C. which, from the freezing point curve, corresponds to a 75 to 85% reaction. The data in Table I call for an 84% reaction under these conditions so that it is evident that the reaction velocity is unaffected, within limits of any rate, by the presence of glass surface or mercury vapor.

of which melt over a convenient temperature range) were placed in the clean dry tubes which were sealed to a manifold thoroughly evacuated and sealed off. They were then placed in a constant temperature air bath (to be described elsewhere) where the capsules burst and the reaction proceeded in the vapor state. After a definite time in the thermostat the tubes were removed and rapidly cooled. The contents were then condensed at -78° C. into the small bulb which was finally sealed off from the main tube. A rough freezing point curve of mixtures of isobutyraldehyde and para-isobutyraldehyde had previously been determined,

### The Energy of Activation

As is apparent from Fig. 1, the straight lines obtained by plotting  $\frac{1}{T}$  against  $-\log k$  (columns Nos. 7 and 8, Table I) for both parabutyrals are close together and almost parallel. That of the iso-compound (Curve III) is a little above and has a slightly greater slope than that of the normal compound (Curve II). The corresponding activation energies are 42,800 and 42,000 calories per mole respectively—quantities identical within the limit of error of the rate measurements and very nearly the same as the previously reported value for paracetaldehyde (Curve I), *viz.*, 44,200 calories. Recent velocity measurements using highly purified paracetaldehyde indicate that this figure may be somewhat too high and that it is probable that all members of the series have the same activation energy. Until more accurate data are available, however, the following equations may be taken as giving the velocity constants of the three compounds already investigated:—paracetaldehyde,  $\ln k = 34.83 - \frac{44,200}{RT}$ ; para-*n*-butyraldehyde,  $\ln k = 33.12 - \frac{42,000}{RT}$ ; para-isobutyraldehyde,  $\ln k = 34.06 - \frac{42,800}{RT}$ .

Other members of the series including mixed and halogenated paraldehydes are being studied.

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## THE DENSITY OF PROPYLENE IN THE LIQUID AND VAPOR PHASES NEAR THE CRITICAL TEMPERATURE<sup>1</sup>

BY C. A. WINKLER<sup>2</sup> AND O. MAASS<sup>3</sup>

### Abstract

The density of propylene in the liquid and vapor phases was measured over the range 66° to 92° C. by a refined dilatometer method and at the higher temperatures at the same time by a float method. Good agreement was found between the two methods, the latter being preferable in the neighborhood of the critical temperature. It was found that at temperatures below the critical, no variation in vapor density accompanied a change in the relative volume of the liquid. The data were obtained principally for an investigation to be published simultaneously on discontinuities above the critical temperature.

In the past few years, the use of propylene in the study of critical and other phenomena has become very widespread, probably owing to the suitable critical constants of this substance, and to its polar molecular nature. Coincidentally, there has been an increased demand for accurate data on the physical constants of this substance. Many of these constants have been determined with sufficient accuracy for most purposes. Data on the density of propylene over a temperature range approaching the critical, in the liquid and gaseous phases, are not available however. Since these data were essential for the calculation of the surface tension reported previously (3), and for other investigations in progress in this laboratory, they were ascertained over a range of approximately 60°–92° C.

The densities of the vapor at several temperatures were determined with the apparatus described elsewhere (4). In so doing, the float was elevated to some position in the bomb above the liquid phase, and the extension of the spiral ascertained in the usual manner. The temperature of the bath was maintained equalized throughout, and constant at any desired temperature to within 0.03° C. The effect of varying the relative volume of the liquid to the total bomb space was investigated, since it was known that at temperatures above the critical a decrease in the space available for propylene was paralleled by an increase of density. It was found, however, that at temperatures below the critical, no significant variation in vapor density accompanied a change in the relative volume of the liquid. Great care was taken in determining vapor densities below the critical temperature to ensure equilibrium between the phases. The values of the density which are recorded represent those obtained for constant readings of the extension during the lapse of at least an hour. It was found possible to obtain values for the density of the liquid phase by the same method, at temperatures not more than approximately 3° C. removed from the critical point. The error in the determination of the vapor density of the liquid very near to the critical temperature should not exceed that involved in the calibration of the float, namely, 0.3%.

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The density of the liquid phase through the temperature range from 60°–90° C. was determined by the dilatometer method. A dilatometer was constructed from Pyrex capillary tubing of approximately 1 mm. bore and 2 mm. wall thickness. A bulb of approximately 0.7-cc. volume was blown at one end. Immediately above this bulb was sealed a finely drawn glass pointer to serve as a zero point on the stem. A bulb of some 1.5-cc. capacity was blown at a distance of 18 cm. from the pointer. The volumes of the bulbs were such that when the dilatometer was filled to a convenient distance above the pointer, the liquid occupied very nearly  $\frac{3}{4}$  of the total volume. This precaution was taken to avoid excessive motion of the meniscus, since it has been found from experience that in a tube containing this ratio of liquid to total available space, the meniscus remained practically motionless as the critical temperature was approached.

The stem of the dilatometer was calibrated by introducing weighed quantities of mercury, and measuring the height of the mercury column, relative to the pointer, with a cathetometer after the addition of each successive portion. The volume was calculated for various positions in the stem, and a calibration curve plotted. The total volume was also ascertained by calibration with mercury.

The dilatometer was thoroughly cleaned and dried, and propylene, prepared by the dehydration of isopropyl alcohol over alumina at 362° C. and purified by low temperature fractionation (as described by Coffin and Maass (1) and Maass and Wright (2) ), was introduced by low temperature condensation, until approximately  $\frac{3}{4}$  of the entire volume was occupied by liquid. The dilatometer was then sealed off just above the upper bulb, and immersed in a bath of glycoline oil, the temperature of which could be maintained at any desired value, constant within 0.03° C., by manual control of resistances in series with the electrical heating elements. The position of the meniscus was ascertained at various temperatures with a cathetometer, with an error not exceeding 0.04 mm., the position in each case being determined with reference to the pointer affixed to the stem of the dilatometer.

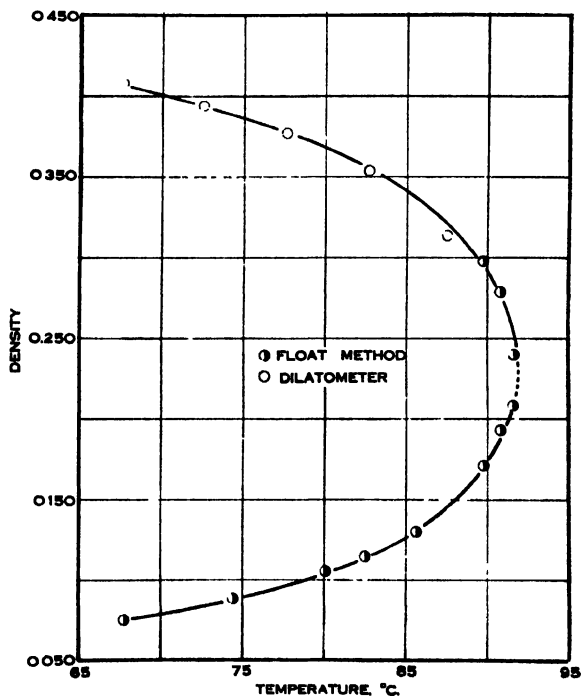


FIG. 1. Densities of vapor and liquid below the critical temperature as determined by dilatometer and "float" methods.



The dilatometer was then carefully cleaned, dried and weighed with its contents. The tip was broken off, care being taken to prevent loss of fragments of glass, and the propylene allowed to evaporate. The dilatometer was then flushed with air and reweighed. From the data obtained dilatometrically, in conjunction with the data for the vapor densities, the density of the liquid propylene at each temperature was calculated. The vapor densities were not determined at the same temperatures as the liquid densities; consequently, those values of the vapor density necessary in the calculation of the densities of the liquid were obtained from the vapor density-temperature curve.

The data obtained for the vapor densities by the float method are set forth in Table I, those for the liquid densities, in Table II. Fig. 1 is a graphical representation of all the data obtained.

TABLE I  
THE DENSITIES OF PROPYLENE VAPOR FROM 67.70° C. TO THE CRITICAL TEMPERATURE

Temp., ° C.	67.70	74.40	80.10	82.50	85.60	89.70	90.80	91.50
Vapor density	0.0747	0.0884	0.1054	0.1150	0.1300	0.1710	0.1927	0.2008

TABLE II  
THE DENSITIES OF PROPYLENE LIQUID FROM 67.60° C. TO THE CRITICAL TEMPERATURE

Temp., ° C.	67.60	72.50	77.60	82.70	87.50	89.70*	90.70*	91.60*
Density of liquid	0.4080	0.3937	0.3771	0.3540	0.3136	0.2983	0.2776	0.2390

\* These values were obtained by the float method; the remainder by the dilatometer method.

### Discussion

These results need little or no discussion, except, perhaps to mention that the density of the liquid determined dilatometrically at 87.5° C. is probably somewhat too low, rather than the values at 89.7° C. being too high. The reason for this conclusion is not far to seek. At temperatures approaching the critical, the meniscus in the dilatometer became very restless, and also quite diffuse, both of which factors contributed to a relatively large experimental error in the case of the value at 87.5° C. The float method, on the other hand, is not subject to these disadvantages.

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# DENSITY DISCONTINUITIES AT THE CRITICAL TEMPERATURE<sup>1</sup>

BY C. A. WINKLER<sup>2</sup> AND O. MAASS<sup>3</sup>

## Abstract

A technique for measuring densities in various parts of a one- or two-component system, raised above its critical temperature, is described. Considerable improvements over a method for this purpose recently described by one of the writers consist in greater flexibility of manipulation and in making possible a variation in the volume of space confining the medium during the experiment. Propylene and methyl ether were the two media examined. From the results the following generalizations regarding aberrations from the continuity of state were found to hold for both.

When either liquid was heated above the temperature at which the visible meniscus disappeared, the density below this point of disappearance was found to be greater than that above. The density was uniform throughout each portion, undergoing a relatively sharp change in the small region where the meniscus was last seen. After one hour of temperature equilibrium, the difference in density between top and bottom became constant and remained unaltered for six hours. Constant stirring or temperature fluctuations of the order of 0.02° C. do not alter this density difference. A decrease in the volume available for the medium increases the density difference between the top and bottom, a continuous relation existing between available space and density difference. With decrease in available space, the densities of both upper and lower portions of the medium increase, the density of the lower more rapidly than that of the upper. These results were reproducible quantitatively in the experiments carried out to date. The density difference for a fixed available space decreases with rise in temperature and is measurable up to at least 10° C. above the critical temperature. A number of miscellaneous experiments are described which form the basis of work now being carried out. The theoretical significance of aberrations from the continuity of state is discussed to the extent warranted by the present stage of the experimental investigations, and tentative conclusions are drawn.

## Introduction

Continuity of state at the critical temperature has been generally accepted until quite recently. O. Maass and coworkers (6, 8, 12) have recently reconsidered the limitations within which this "continuity" may be said to hold. The incentive to this reconsideration was the observation by Sutherland and Maass (7) of a marked discontinuity of the velocity of reaction near the critical point. Earlier work, notably that of Cailletet and Hautefeuille (1), Galitzine (4), Traube (10), Teichner (9), Callendar (2), has pointed qualitatively to the existence of a discontinuity of properties at the critical temperature. The evidence adduced from these investigations was based, however, on experiments to which rather serious objections may be taken. Furthermore, the investigations were all made on one-component systems. The discontinuity observed by Sutherland and Maass (7), on the other hand, was the result of an investigation of a two-component system. Work was planned, therefore, to establish the applicability of the continuity postulate to systems of more than one component, or, conversely, the existence of a definite discontinuity

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in one-component systems. As a preliminary to the more detailed investigation, the pressure-temperature relations in two-component systems, comprised of the substances to be investigated, were studied (11).

Although investigations of two-component systems were definitely planned, work in progress at the time showed the desirability of developing an apparatus and technique which would also be applicable to one-component systems. The determination of the surface tension of methyl ether and propylene by Winkler and Maass (12) pointed to a definite discontinuity in one-component systems, at the critical point, as did also an investigation undertaken later by Morris and Maass (6) on the sorption of propylene on alumina in the critical region. An investigation of the discontinuity of density in a one-component system by Tapp, Steacie and Maass (8) also pointed quite definitely to the fallacy of the continuity hypothesis.

Tapp, Steacie and Maass (8) showed that, in the case of methyl ether, when a tube was filled with a mass which would show a rise or fall of the meniscus with temperature change, a definite density difference persisted in the medium above the so-called critical temperature. These authors showed that this difference persisted at temperatures above the critical in spite of stirring for indefinitely long periods of time. A most interesting fact observed was that the difference in density was in the form of a discontinuity in that portion of the medium where the meniscus had disappeared, thus showing that the observations could not be explained on the bases of a gravitational effect or high compressibility of the medium at the critical temperature. Unexpectedly, it was observed that a temperature gradient in the bath, the top being slightly cooler than the bottom, eliminated the discontinuity or density difference.

It was found also that reproducible results were obtained for each particular bomb investigated. The actual magnitudes of the difference varied from one bomb to another, and could not be related owing to the lack of exact information concerning the mass-volume relations in the various bombs. In other words, the existence of a persistent density difference above the critical temperature in the case of a liquid was established qualitatively. It is, of course, recognized that these liquids may possibly be contaminated by traces of catalysts. It obviously became necessary to carry out the investigations in such a way that quantitative, and not merely qualitative, relations could be established, before any theoretical deductions concerning the nature of the liquid and gaseous states could be made. It was for this reason that the work described in the present paper was undertaken.

Although, as previously stated, the original intention was to investigate two-component systems, the apparatus designed for such systems having been completed when the first results of Tapp, Steacie and Maass were obtained, full attention was directed to one-component systems in an endeavor to establish the experiments on a quantitative basis. This was done only because the flexibility of the apparatus described herein makes possible numerous experiments and a high degree of efficiency, which cannot be

obtained in the type of apparatus described by Maass and coworkers. With the apparatus developed by the authors, it has been possible, not only to confirm the results of Tapp, Steacie and Maass (8), but also to establish the experiments on a quantitative basis, and add considerably to the present knowledge of one-component systems. As will be evident, the experimental technique has been greatly improved. In spite of this, however, the accumulation of essential data took considerable time. While it would have been highly desirable to have suspended publication until an incontrovertible number of data had been obtained, the award of a Rhodes scholarship to the junior author necessitated his discontinuance of the problem, and made imperative publication of the results obtained to date.

Apart from the investigation of factors governing discontinuity of density above the critical temperature, the densities of liquid propylene and its equilibrium vapor were measured over a considerable range of temperature up to the critical. These data were necessary for the calculation of the surface tension of propylene reported by Winkler and Maass (12), and also in the investigation of sorption in the critical region by Morris and Maass (6). Aside from this, the data serve to show the splendid agreement obtained between densities measured by means of the McBain-Bakr quartz spiral balance and those determined dilatometrically.

## Experimental

### *Apparatus*

In its essential aspects, the apparatus is represented in Fig. 1. Two steel bombs, constructed and assembled as outlined by Winkler and Maass (11), were connected to a Cailletet pump in the manner described. A heavy-walled Pyrex glass tube, some eight feet in length, having a U-bend as shown in the diagram, was sealed at one end to a glass bell in the second steel bomb. The glass portion of the assembly was effectively sealed to the metal portion as previously described (11).

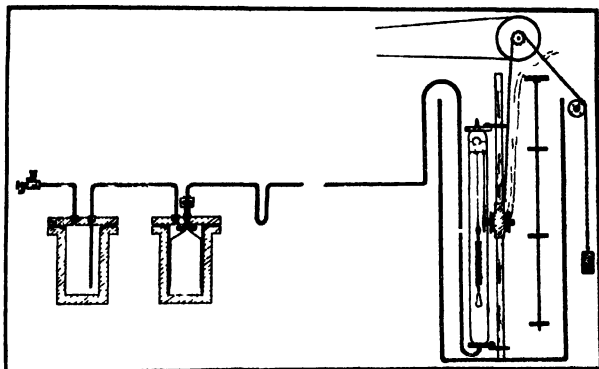


FIG. 1. Diagram of apparatus for measuring densities above critical temperatures.

A glass bomb was constructed from Pyrex tubing of 1.5 cm. internal diameter and 4 mm. wall thickness. The tubing was selected with special reference to its optical properties and freedom from apparent 'flaws'. A length of capillary tubing was sealed to one end, and bent so as to pass close to the wall of the bomb, and extend slightly beyond its upper end.

The float, glass pulley and counterweight (consisting of a wire nail in a glass sheath) were similar to those used by Tapp, Steacie and Maass (8).

The volume displacement of the float, with hook attached, was determined with a pycnometer. The weight of the float was also determined and from these data the effective density was calculated. The error in the calibration of the float was less than 0.3%.

A silk thread, fastened to the hook on the sheath surrounding the nail, was passed over the pulley, and attached to a glass counterweight. The thread passed through small glass loops sealed to the under side of the short glass tube carrying the pulley. One of these was located along a diameter of the tube, and served to maintain the end of the thread which carried the counterweight centrally in the bomb. The other loop was fixed to the periphery of the tube, and served to prevent the nail from swinging towards the central portion of the bomb.

To the bottom of the counterweight was attached a machine-wound, calibrated quartz spiral. The fibre constituting the spiral was approximately 0.08 mm. in diameter, the spiral itself being 4.305 cm. long (the "normal" length), with a diameter of approximately 0.5 cm. The spiral was calibrated by suspending it from a rigid support, attaching weights of varying magnitude to the lower hook of the spiral, and determining with a vertical cathetometer the total length corresponding to each weight. The normal length, *i.e.*, with no weight attached, was also determined. From these data the sensitivity of the spiral was calculated by dividing the weight in grams by the corresponding extension in millimetres. The sensitivity was found to be 0.00512 gm. per mm. and practically independent of the load imposed, except for very small extensions. Fatigue of the spiral under stress does not occur over a period of as much as six months.

The assembly, comprised of the float, spiral, and the nail and sheath with their counterweight, suspended from the pulley, was carefully inserted into the glass bomb, and allowed to rest on diametrically opposite indentations in the wall of the bomb. A short piece of capillary was then sealed to the upper end. To prevent destruction of the silk thread suspension, due to conduction and radiation of heat while drawing down the tube and effecting the seal, the bomb was immersed in water to a point slightly above the location of the pulley. The unit was then sealed to the capillary leading to the system of steel bombs and Cailletet pump, in the manner indicated by the diagram.

The wall of the float was relatively thin; the float was therefore tested for possible changes in effective density due to compression at high pressures. It was attached to a quartz spiral, and suspended in a water-filled Pyrex glass bomb, connected to the Cailletet pump. A small weight, sufficient to cause submergence, was attached to the float. After sealing off the bomb, various pressures were applied, and the extension of the spiral determined at each pressure with a cathetometer. Duplicate determinations showed no change in the extension of the spiral, even at pressures up to 60 atm. Since

the compressibility of water is negligible at pressures of this magnitude, it was concluded that no significant variation in the effective density of the float would occur over the range of pressures to be encountered. The test also proved that the float was capable of withstanding the required pressure without collapsing.

Motion of the spiral and float within the bomb was effected magnetically. A solenoid, capable of carrying 10 amperes, and provided with an iron core, was mounted on a carrier which could be moved vertically on a grooved brass rod, which also carried adjustable supports for the glass bomb. By suitably arranging the magnet relatively to the bomb, the core was maintained at a distance of approximately 2 mm. from the wall of the bomb throughout its length. True vertical positions of both the brass rod and the bomb were obtained by plumb lines.

To facilitate moving the magnet, a chain connected to the carrier was passed over a toothed wheel, and a counterweight suspended from the other end. On the same axle as the toothed wheel was fixed a large pulley, from which a belt passed to another pulley some eight feet distant. This enabled the magnet to be moved by the operator at a distance from the bath.

The thermostat contained three gallons of dibutyl phthallate. This liquid was much superior to various oils which have been used, since it undergoes no apparent decomposition at the temperatures employed in this investigation. Three electric heaters, of 500 watts capacity each, and individually regulated by manual control of external resistances, were installed. These were located at as great a distance from the bomb as available space in the bath permitted, two passing down the side of the container, and one being located near the surface of the liquid. The rate of stirring could be regulated at will.

Since temperature control was of fundamental importance and the existence of a temperature gradient between the top and bottom of the thermostat must be avoided, a copper-constantan thermocouple was placed in the bath. Four junctions were located near the top of the bomb, and four near the bottom. It was connected to a sensitive galvanometer, placed at a distance of approximately  $1\frac{1}{2}$  metres from a scale. A deflection of 1 cm. on the scale resulted from a temperature difference of approximately  $0.02^{\circ}$  C. between the top and bottom of the bath. When desired, a temperature difference could be maintained or eliminated by suitable alterations in the current passed through the heaters. Temperatures were ascertained with a standardized thermometer, with an error not exceeding  $0.03^{\circ}$  C.

Changes in the extension of the quartz spiral were determined with a cathetometer, with an accuracy of 0.02 mm. To facilitate observation, a microscope objective (16 mm.) was fitted into the optical train. All readings were made to include the loops at the ends of the spiral.

Since shattering of the glass bomb at high pressures was a possibility, precautions similar to those described previously (8) were taken to protect the operator.

### *Preparation and Purification of Materials*

Methyl ether was prepared in the manner indicated by Winkler and Maass (11). Propylene was prepared by the dehydration of isopropyl alcohol over alumina at 362° C., and purified by low temperature fractionation, as previously mentioned (3, 13). Since impurities might have a large influence on the nature of the phenomenon at the critical temperature, great care was taken to obtain a maximum degree of purity with the facilities available. Dehydration was thorough, and the low temperature fractionations were carried beyond the point at which the substances were ordinarily deemed of sufficient purity for other purposes. Constancy of vapor pressures and critical temperatures were taken as criteria of the purity. Only when these physical constants showed no change upon successive distillations were the substances considered of sufficient purity for the purpose of this investigation.

### *Manipulation of the Apparatus*

Although the manner in which the apparatus was applied to the investigation of various problems relating to density will become more evident by the presentation of the results obtained, a few general remarks should be made at this point.

Prior to the introduction of the liquid which it was desired to investigate, mercury was pumped from the steel bombs to some point along the capillary connecting the glass bomb to the Cailletet pump assembly. By freezing the mercury at the U-bend in this capillary with a mixture of solid carbon dioxide and acetone, the bottom of the bomb was virtually sealed. When desired, however, the mercury seal could be permitted to liquefy, and the volume of the space available for the liquid under investigation readily changed by altering the position of the mercury in the capillary, or in the bomb itself, by manipulation of the pump.

To introduce the liquid into the glass bomb containing the quartz spiral assembly, the short capillary at the top of the bomb was sealed directly to the low temperature fractionating unit in which the liquid was prepared. A mixture of solid carbon dioxide and acetone was then introduced into the thermostat, and the liquid permitted to distil into the bomb, which had previously been evacuated and flushed several times with vapor of the liquid under investigation. The quantity of liquid introduced was ascertained by effecting the last distillation from a graduated tube. When the desired amount of liquid had been condensed into the bomb, the top capillary was drawn off. The carbon dioxide-acetone mixture was then removed from the thermostat, and the dibutyl phthallate introduced. In actual practice a small electric heater was fitted over the top capillary, since this protruded above the level of the thermostat fluid. When desired, the temperature of the capillary could be maintained above that of the remainder of the bomb, or, by shutting off the heater, the capillary served as a region for condensation of the liquid in certain experiments in which this was desirable.

Prior to carrying out an experiment, the zero point of the thermocouple was adjusted by shorting the galvanometer with a copper wire directly across the terminals. A long wire was avoided, since there was a possibility of temperature differences over the greater distance which would result in a slight thermoelectric current, with a consequent false zero adjustment. It might be argued that, since the thermocouple was permanently located, with junctions at both ends of the bomb, there was no definite assurance that there did not exist unascertained temperature variations in portions of the bath between the thermocouple junctions. To insure that such differences did not exist, and to act as a check on the thermocouple employed, a second thermocouple was inserted into the bath. The upper junctions of this thermocouple were flexible, which enabled any portion of the bath to be explored at will.

The incorporation of an expansion chamber, consisting of a short length of bomb tubing, in the capillary line between the mercury seal and the glass bomb, assisted materially in manipulation.

A blank run was made to determine whether or not the three-gallon Pyrex jar containing the thermostat fluid, or the glass bomb itself, produced any optical aberration. The extension of the spiral was measured at centimetre intervals in the bomb, the liquid being contained in the expansion chamber in the capillary line. The temperature was held constant. In this way, the float was in the vapor phase, of constant density, throughout the series of measurements. Any inequalities in the length of the spiral were noted, and corrections applied to all subsequent readings for corresponding positions in the bomb. This aberration was never large, but in some positions was in excess of the experimental error. As will be seen later, a correction was applied for such positions.

## Results and Discussion

### *Preliminary Work (with Methyl Ether)*

The apparatus having been assembled at the time Tapp, Steacie and Maass (8) were obtaining results with the apparatus described by them for one-component systems, it was deemed advisable to confirm their results qualitatively in an independent manner. Methyl ether was introduced, therefore, into the bomb, and experiments similar to those previously described were conducted. In general, the results obtained were in entire agreement qualitatively with those obtained by the above authors. It was found that a definite density difference persisted in the medium at temperatures above the critical, in positions above and below that at which the meniscus disappeared. Neither extreme care in equalization of the temperature in the bath, nor vigorous stirring of the medium eliminated the difference in density. Evidence was obtained also that time alone did not govern the magnitude of the density difference, a constant difference obtained after approximately 45 min. persisting for an hour or more. Increase of temperature resulted in a decreased density difference in the upper and lower portions of the tube, but the difference persisted at temperatures considerably above the critical.



To economize space, the results obtained in the preliminary studies are not recorded. Minor discrepancies observed were found to be due to lack of experience with the apparatus, and the results obtained later were much more reliable.

### Propylene

Propylene was introduced into the bomb, in place of the methyl ether. This was done for two main reasons: first, to obtain data necessary for the calculation of the surface tension of propylene, reported by Winkler and Maass (11); second, to determine whether or not propylene behaved similarly to methyl ether in exhibiting a definite difference in density above and below the position at which the meniscus disappeared, at temperatures above the critical. The data obtained for the vapor density of propylene over a temperature range from 67.70° C. to the critical point, and the density of liquid propylene very near the critical temperature, are set forth elsewhere (13). It should be pointed out that while obtaining these data, the propylene was permitted to occupy the entire volume of the bomb.

In the following, the mass-available volume is defined by the volume, in the bomb above the mercury, available for the propylene, irrespective of whether it is liquid or gaseous.

Maintaining the mass-available volume relation the same as stated above, the temperature was raised above the critical, which was determined as 92.0° C., and held constant at 93.40° C. The medium was vigorously stirred by raising and lowering the spiral assembly in the intervals between taking

readings: Time is not recorded, since readings of the extensions were taken in all cases until no variation was noticeable over a period of half an hour in the density at any given position in the bomb. The temperature was equalized throughout the bath. The results are shown in Table I.

TABLE I

DENSITY OF PROPYLENE ABOVE AND BELOW POSITION  
WHERE MENISCUS HAD DISAPPEARED

Temp., ° C.	$d$ (top)	$d$ (bottom)
93.40	0.2056	0.2142
95.40	0.2091	0.2135
98.40	0.2097	0.2117

These data, in conjunction with those mentioned (13) are graphically represented in Fig. 2. The striking dissimilarity between the parabolic curve (13, p. 611) which represents the classical continuity hypothesis, and the curve shown in Fig. 2 of this paper, illustrates the marked discontinuity which has been observed consistently throughout the present investigation. It should be emphasized, perhaps, that the magnitude of the difference in density *above* the critical temperature has been shown by later work to depend on the relative space available for the medium in the bomb. Below the critical temperature, however, it was experimentally determined that the relative volume of liquid present had no ascertainable influence on the vapor or liquid densities. Since the data represented in Fig. 2, both above and below the

critical temperature, were obtained with the same relative volume of liquid in the bomb, it is justifiable to compare the two types of curves, irrespective of the absolute magnitude of the density difference observed above the critical temperature.

That propylene behaved in a manner similar to methyl ether was further shown by determining the density in various positions in the bomb. The relative volume of liquid to total available space was constant (though not the same as previously described); the temperature was also maintained constant and equalized at 93.40° C. The float was moved to various positions in the bomb, and the densities at these positions ascertained. The meniscus disappeared at a reading of 28.5 cm. on the cathetometer.

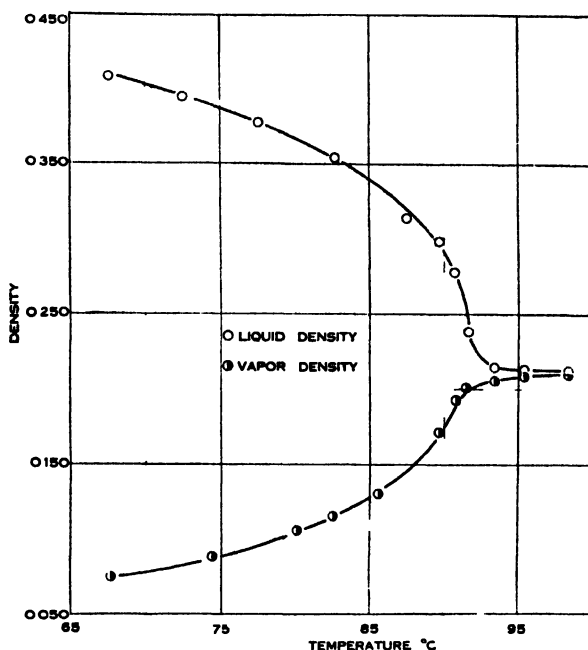


FIG. 2. Extension of classical density vapor-liquid curve above critical temperature.

TABLE II

RELATION BETWEEN POSITION OF FLOAT AND DENSITY OF PROPYLENE

Position of float, cm.	19.875	20.350	26.020	27.850	31.870	35.060
Density, gm./cc.	0.2142	0.2145	0.2145	0.2150	0.2056	0.2056

NOTE.—Temp., 93.40° C.

It will be observed that the discontinuity in density occurs in the region where the meniscus disappeared. A more detailed study of this phase of the problem is given later.

At this juncture it was necessary to clean the glass bomb, owing to slight impurities from the mercury having been left on the internal wall. That this was possible serves to illustrate very markedly the flexible nature of the apparatus. The silk thread was drawn up through the opened capillary at the top, after which, by very careful manipulation, the bomb was cleaned with chromic acid, water, alcohol and ether, and thoroughly dried. The bomb was removed from the bath for this purpose.

The bomb was again sealed into place, and a freshly prepared sample of propylene distilled into it. It was the intention to determine whether or not

a definite relation existed between the density difference and the relative volume of propylene to total bomb space. For this purpose, the meniscus was set at various positions in the bomb, this position being ascertained on the cathetometer.

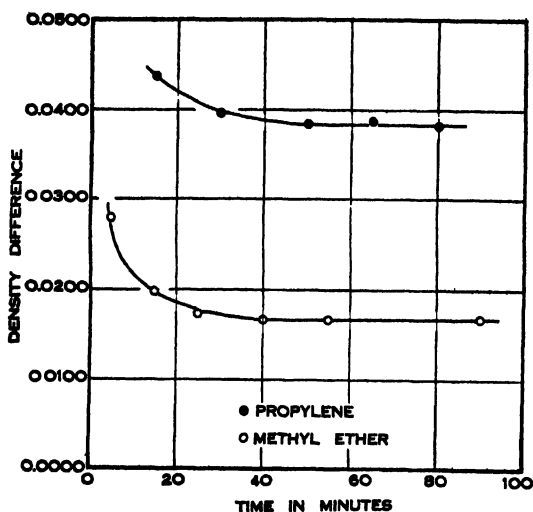


FIG. 3. Relation between time and density equilibrium at constant temperature.

The densities at the top and bottom of the bomb were determined for each position, readings being taken at definite intervals of time until constant values were obtained. A typical time-density curve for propylene is shown in Fig. 3. The meniscus was first set at 23.3 cm., and the density difference observed was 0.0299. With a setting at 25.5 cm., the difference was 0.0382, and at 26.5 cm., 0.0418. All positions were recorded at room temperature, experience having shown that variations of as much as 10° C. did not appreciably alter the position at temperatures of approximately 30° C. The essential point to notice is the marked and regular increase in the density difference as the available space in the bottom was reduced by the introduction of mercury. The relation between the density difference in positions above and below that at which the meniscus disappeared, and the relative volume of propylene to total bomb space is shown in Fig. 4. This relation will become more evident in results to be presented later.

Unfortunately, before the investigations with propylene could be continued, the silk thread slipped off the pulley in the bomb. This necessitated opening the capillary at the top of the bomb, with consequent loss of the propylene. With the aid of a fine, hooked wire, it was relatively easy to repair the apparatus. Methyl ether being available at the time, however, and time being limited in which to pursue further investigations, it

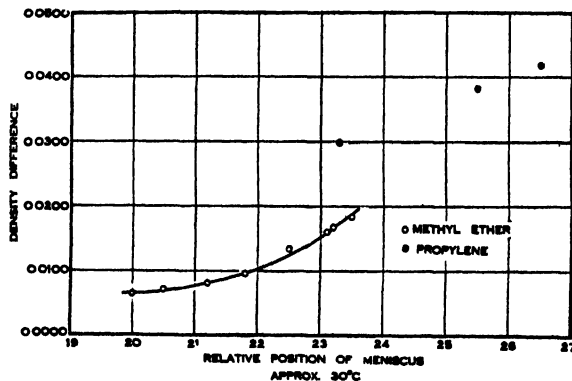


FIG. 4. Relation between density difference between upper and lower portions of medium, and relative volume of bomb space, as given by position of meniscus at room temperature.

was deemed advisable to use methyl ether for other experiments which had been planned. All data presented hereafter, therefore, refer to methyl ether as the medium.

### Methyl Ether

The first study undertaken was the influence of time on the attainment of equilibrium values of the density above and below the position at which the meniscus disappeared. The meniscus was adjusted to a position ascertained as 23.2 cm. on the cathetometer. The temperature was maintained constant

TABLE III  
INFLUENCE OF TIME ON DENSITY DIFFERENCE

Time, min.	$d$ (bottom)	$d$ (top)	Density difference
5	0.2618	0.2339	0.0279
15	0.2592	0.2394	0.0198
25	0.2568	0.2394	0.0174
40	0.2568	0.2401	0.0167
55	0.2568	0.2401	0.0167
90	0.2568	0.2401	0.0167
120	0.2568	0.2401	0.0167

and equalized throughout the bath at 128.8° C., i.e., 1.8° C. above the critical temperature as defined by the disappearance of visually apparent inhomogeneity in the medium. Values of the density at the top and bottom of the bomb were determined at definite time intervals. The results are shown in Table III and graphically represented in Fig. 3.

The data are in very good agreement with those of Tapp, Steacie and Maass (8), and show that equilibrium is established after approximately one hour. Stirring the contents of the bomb by raising and lowering the spiral assembly numerous times had no effect on the density difference finally established.

A study was next made of the relation between the difference in density observed in positions above and below that at which the meniscus disappeared, and the relative volume of the bomb occupied by the liquid. As a measure of this relative volume, the position of the meniscus at approximately 30° C. was assumed. This position was set to various values, and the densities determined in the usual manner at various positions in the tube. In no case was equilibrium assumed to be established in less than two hours, which is twice

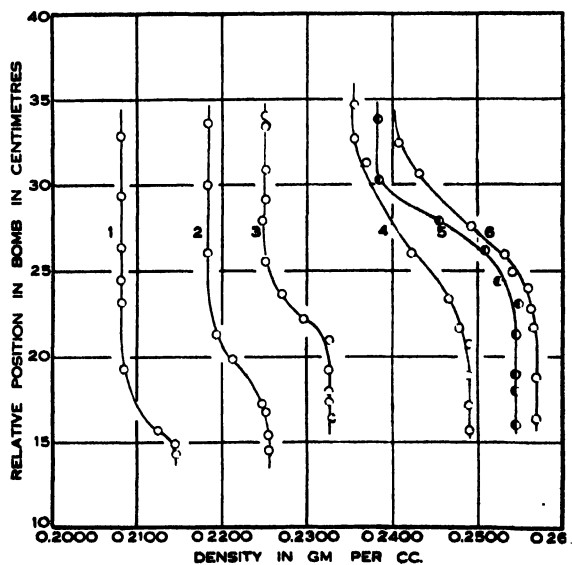


FIG. 5. Relation between density and position in bomb for various bomb spaces as given in Table IV. Relative meniscus position (room temperature)—Curve 1, 20.0 cm.; Curve 2, 20.5 cm.; Curve 3, 21.2 cm.; Curve 4, 22.5 cm.; Curve 5, 23.1 cm.; Curve 6, 23.3 cm.

the time indicated by the time-density curve. Stirring inside the bomb was vigorous, and the temperature equalized in all cases. The results obtained are set forth in Table IV and graphically represented in Fig. 5.

TABLE IV

RELATION BETWEEN FLOAT POSITION AND DENSITY CORRESPONDING TO RELATIVE VOLUME OF BOMB OCCUPIED BY THE LIQUID AS INDICATED BY MENISCUS POSITION AT 25° C.  
MEASUREMENTS ALL MADE AT 2° ABOVE THE CRITICAL TEMPERATURE

*A = 20.0 cm.; B = 15 cm.		A = 20.5 cm.; B = 16 cm.		A = 21.2 cm.; B = 20 cm.		A = 21.8 cm.; B = 23.5 cm.	
Float position	Density	Float position	Density	Float position	Density	Float position	Density
14.300	0.2145	14.525	0.2254	16.480	0.2329	15.610	0.2469
14.925	0.2145	15.370	0.2254	17.420	0.2326	19.725	0.2466
15.710	0.2125	16.765	0.2252	17.995	0.2326	20.430	0.2463
19.295	0.2084	17.325	0.2247	19.320	0.2326	22.835	0.2466
23.170	0.2081	19.825	0.2213	20.965	0.2326	25.450	0.2435
24.465	0.2081	21.335	0.2193	22.210	0.2296	26.145	0.2415
26.405	0.2081	26.080	0.2183	23.715	0.2271	28.010	0.2380
29.440	0.2081	29.945	0.2183	25.560	0.2251	29.850	0.2377
32.915	0.2081	33.600	0.2183	27.925	0.2248	30.855	0.2373
				29.215	0.2251	33.425	0.2370
				30.920	0.2251		
				33.530	0.2251		
				34.050	0.2248		
A = 22.5 cm.; B = 24.0 cm.		A = 23.1 cm.; B = 24.0 cm.		A = 23.25 cm.; B = 24.50 cm.			
Float position	Density	Float position	Density	Float position	Density		
15.675	0.2490	16.000	0.2544	16.385	0.2568		
17.210	0.2490	18.220	0.2544	18.750	0.2568		
18.930	0.2490	18.990	0.2544	21.645	0.2565		
20.695	0.2490	21.275	0.2544	22.830	0.2562		
21.590	0.2483	23.120	0.2548	24.020	0.2559		
23.390	0.2466	24.350	0.2524	24.855	0.2540		
26.025	0.2421	26.225	0.2509	26.010	0.2531		
31.245	0.2370	27.935	0.2455	27.560	0.2493		
32.770	0.2356	30.340	0.2384	29.120	0.2452		
34.650	0.2356	33.860	0.2384	30.690	0.2432		
				32.465	0.2408		

\* A = position of meniscus at 25° C.; B = approximate position of meniscus at the critical temperature.

Perhaps the most evident feature of all the curves obtained is the characteristic discontinuity in density in the portion of the medium where the meniscus was last visually evident. This fact is in entire agreement with the results obtained by Tapp, Steacie and Maass (8), and with those noted previously in this paper where propylene was the medium. The family of curves also exhibits a feature which was not noticed previously, owing to the unavoidable differences in size of bomb tubing, and other variations. From the present work it is evident that an increase in the space available for the medium

results in a sharper discontinuity, as indicated by density measurements. The curve obtained when the meniscus was set at 22.5 cm. represents values of the density taken after six hours continued running at 128.8° C. with the temperature equalized throughout the bath during the whole period. This was done in an effort to eliminate the density difference due to time alone. Stirring was frequent and vigorous. No significant decrease in the difference occurred after a period of approximately one hour.

Tapp, Steacie and Maass (8) found that for one bomb no density difference prevailed above the critical temperature. This was tentatively attributed to the particular amount of liquid contained in the bomb, such that the meniscus remained practically in its original position throughout the rise in temperature. For a rising or falling meniscus, however, a density difference was observed above the critical temperature. It was of interest, therefore, to determine whether or not the density difference fell to a minimum value for some particular position of the meniscus. The curves represented in Fig. 4 serve to provide this information. From a consideration of the curves, there seems to be no indication of other than a continuous variation of density difference for different relative mass-available volume relations.

The regularity of the relation between density difference and the available space in which the medium is contained is emphasized by a plot of the values of the difference in density against the positions of the meniscus at approximately 25° C. The trend of this relation is shown in Table V and graphically represented in Fig. 4.

TABLE V

RELATION BETWEEN THE DENSITY DIFFERENCE ABOVE THE CRITICAL TEMPERATURE AND THE POSITION OF THE MENISCUS AT APPROXIMATELY 30° C.

Position of meniscus, cm.	20.0	20.5	21.2	21.8	22.5	23.1	23.2	23.5*
Density difference	0.0064	0.0071	0.0081	0.0096	0.0134	0.0160	0.0167	0.0184

\* A two-point run—above and below position at which the meniscus disappeared.

Throughout the range of positions investigated, no indication of a minimum in the curve is to be noticed. This fact leads at once to the conclusion that the tentative explanation advanced by Tapp, Steacie and Maass (8) to account for a zero difference of density in the particular bomb mentioned is erroneous.

The regularity of the relation between the density difference and the original position of the meniscus establishes an additional very important fact; namely, that the problem of discontinuity of density in one-component systems is capable of quantitative elucidation. The variations in the densities in the upper and lower portions of the media in the tube can be plotted separately against the amount of available space. This is shown in Fig. 6, where the available space is represented by the position of the meniscus at room temperature. The upper and lower portions, defined by the line where

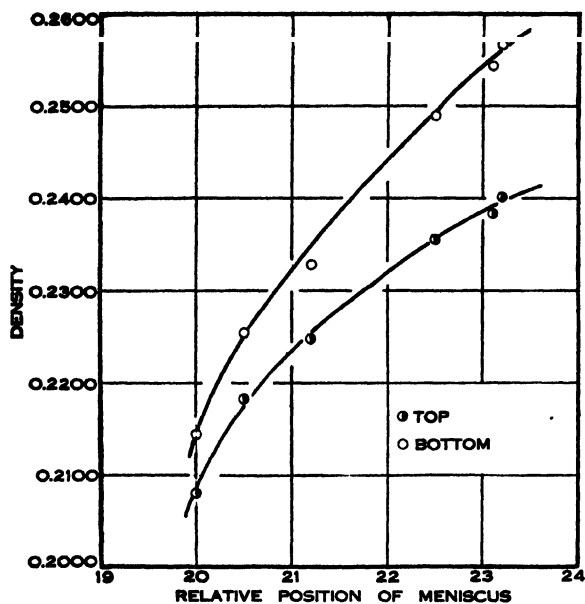


FIG. 6. Relation between density and bomb space for each "phase" existing above the critical temperature.

### Miscellaneous Experiments

A number of miscellaneous experiments were undertaken, the results of which are worth recording and which will form the basis of further careful investigation. In the first place, in the case of methyl ether it was shown that the difference in density decreases by approximately one-half, for every  $2^{\circ}\text{C}.$  rise above the critical temperature. In the case of propylene the decrease is less rapid and density differences were measured as high as  $10^{\circ}\text{C}.$  above the critical temperature.

Both in the case of methyl ether and propylene, the density difference can be destroyed. For instance, after recording a density difference of 0.0160 for methyl ether at  $2^{\circ}$  above the critical temperature, a cold spot was brought into contact with the upper part of the bomb. After 20 min. the medium was of uniform density when kept at constant temperature for several hours. This is in agreement with observations previously recorded (8), which were further confirmed by the following. When the temperature of the upper portion of the tube was kept  $0.1^{\circ}\text{C}.$  below the temperature of the lower portion, the density difference was destroyed. This held for propylene and methyl ether. The density of the lower portion was much more susceptible to change with temperature than the density of the upper portion.

After a density difference had been established at a given temperature  $T_1$  above the critical, and then reduced by raising the temperature to a higher temperature  $T_2$ , then on returning to  $T_1$  the former density difference at  $T_1$  is not re-established.

the meniscus was last seen, behave as separate phases. It must be mentioned that although the data presented in Table IV are given in the order of decreasing available space, the actual determinations were not made in this order. In spite of this, the points fitted on the regular curves shown in Figs. 4 and 5. Furthermore, in the majority of cases, when a certain available space was reproduced by a proper adjustment of the mercury, the densities of the upper and lower portions were also reproduced. Many of the points on the curves given in Fig. 3, were checked repeatedly, after intervening cooling to room temperature.

If a medium was brought to a temperature  $T_1$  above the critical temperature, and then cooled down to below the critical temperature, it was always found that the meniscus reappeared at a markedly lower point in the tube than its initial position. Subsequent heating of the medium to  $T_1$  resulted in a density difference at the point where the meniscus had originally disappeared in the first heating. A further but less sharp density discontinuity was found to exist at the lower point where the meniscus had disappeared on the second heating—but this latter seemed to become less with stirring.

### Conclusions

Having summarized the generalities which may be deduced from a consideration of the data which have been obtained in this investigation, supported by data obtained by one of the writers (8) with a different type of apparatus, it is possible to speculate to some extent on the nature of the phenomenon, and to suggest a few bases upon one of which a possible explanation may be evolved when more data have been accumulated. As pointed out previously (8), the explanation is not likely to be found by a consideration of the influence of gravity; nor is it to be sought in a temperature gradient in the thermostat. That there is an actual persistence of the liquid phase above the critical temperature is possible; that the density differences observed are due to a lag in equilibrium, accentuated by the presence of traces of impurities, is also a possibility; that this lag is due to the viscosity of the medium does not seem possible, in the light of experimental data. On the other hand, it is conceivable that the equilibrium lag exists.

Of the actual persistence of the liquid phase, little can be said with any degree of assurance. Many more data are necessary before such a far-reaching, almost radical explanation can be given for the phenomenon without considerable mental reservation. It is true that the experiments which indicate the hysteresis effect, and the null effect of mechanical stirring, point to some such explanation. But if this is the explanation, the question immediately arises, why is the irreversibility noticed once the difference in density is eliminated? Again, is the irreversibility only apparent, or is it independent of a time lag? Another question which arises in this connection; is the elimination of the density difference, brought about by artificially maintaining a temperature gradient in the bath, due solely to agitation in the medium as a result of a directional flow of the molecules? If so, why does not mechanical agitation over prolonged periods of time bring about a similar elimination of the difference? If the elimination of the difference by a temperature gradient is not due to agitation of the medium, to what is it due? These, and many other similar questions indicate the complexity of the problem.

In dealing with the explanation based on the assumption that there is a lag in equilibrium, it might be said at once that, even assuming this lag to be the underlying cause of the density differences, the explanation does not, in reality, account for the facts observed. Presupposing a lag, the question



arises, to what is the lag due, and in what mechanistical feature of the critical phenomenon is it to be found? If the lag is accentuated by the presence of impurities (these have been computed to be less than 0.02%), how is this accentuation brought about, and how is it operative in maintaining density differences over prolonged periods of mechanical agitation, whereas these differences are not maintained when a temperature gradient exists in the medium? From these considerations alone, it is evident that to explain the observed facts on the basis of a lag in equilibrium due to the presence of impurities is virtually not to explain them at all.

There remains the possibility that the lag in equilibrium, if existent, is a function of time only. Although density differences persisted over periods of as much as six hours, at equalized temperatures, there is the possibility that during a greater time interval, the differences would be eliminated. This speculation is not entirely without theoretical basis. If the classical parabolic density-temperature curve is considered, certain facts are evident. For the liquid, the rate of change of density with temperature, up to the critical point, is given by a negative ratio; hence, the rate of change of volume with temperature,  $dV/dT$ , is positive, and at the critical point would become equal to plus infinity. On the other hand, the rate of change of vapor density with temperature, as the critical point is approached, is given by a positive ratio; hence the rate of change of volume in this case,  $dV/dT$ , is negative, and at the critical temperature would become equal to minus infinity. Now, if the relation between two variables is such that the tangent to the curve representing this relation approaches either plus or minus infinity, it is generally true that some factor governing the relation approaches either zero or infinite value. Is this analogy applicable to the consideration of density differences, with the time factor, governing the relation between density and temperature near the critical point, becoming an infinite quantity? From a consideration of the pressure-volume isotherms at the critical point, an abstruse indication of a similar nature is discernible. At the critical point, the rate of change of volume with pressure,  $dV/dP$ , is mathematically equal to infinity, since the tangent to the curve at this point is perpendicular, or practically so, to the volume axis. Actually, however, the volume does not change infinitely with a change in pressure. Is there, then, some significance to be attached to  $dV/dP$  other than that generally conceived?

The experimental results of greatest interest are that differences in density existing above the critical temperature in a medium are defined by the position where the meniscus disappeared, the density above and below being uniform, dependent on the mass volume, and not influenced by long and thorough stirring. There appear to exist above the critical temperature two well-defined phases. That the lower or "liquid phase" is metastable is possible and in view of some of the experiments probable. Various explanations of mechanism suggest themselves.

The liquid state may be associated with a condition of regional orientation and the critical temperature merely that at which the meniscus or inter-

mediate layer broadens out so that the demarcation between the phases becomes invisible. The liquid phase may consist of aggregations of molecules which persist and these aggregations may be quite large. The "liquid phase" may be disintegrated by evaporation brought on by a distillation when a negative temperature gradient occurs upwards. These and a number of other tentative explanations offer themselves. Apart from a repetition and extension of the measurements described in this paper, viscosity, dielectric constant and refractive index measurements are planned or under way. When these have been carried to completion some of the questions put in this last section may be answered.

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## THE EFFECT OF HIGH-FREQUENCY CURRENTS ON THE TRANSITION POINT OF SUPERCONDUCTORS<sup>1</sup>

BY E. F. BURTON<sup>2</sup>, J. O. WILHELM<sup>3</sup>, A. PITT<sup>3</sup> AND A. C. YOUNG<sup>4</sup>

### Abstract

The present paper represents a continuation of the work on superconducting phenomena for high-frequency currents and the interaction of high-frequency and direct currents. It is found for thin films of tin that there is no appreciable change in the transition point for frequencies from zero up to  $3 \times 10^7$  cycles per sec. There is no effect on the high-frequency point due to direct currents or on the direct current point due to high-frequency currents, when the value of these currents is below about 20 milliamps. There is a very appreciable effect on the direct current point when high-frequency currents up to 200 milliamps. are superimposed on the direct current. This effect varies with the strength of the high-frequency current and not, as thought before, with the ratio of the high-frequency to the direct current.

Several papers (1, 2, 3) have been published by the group of workers in the Cryogenic Laboratory of this Department on the superconducting temperature of various metals for alternating currents of high frequency and also on the effect of alternating currents on the d-c. superconducting temperature and conversely, on the effect of the presence of direct currents on the a-c. transition point.

Among other results reported, there were indications that the transition point (or critical point), by which is meant the temperature at which the resistance begins to change abruptly, for a given wire, was lower when measured for high-frequency currents than when measured for direct currents. Results for frequencies running from  $0.208 \times 10^7$  to  $1.61 \times 10^7$  cycles per sec. indicated that there might be a limiting frequency, about  $10^9$  cycles per sec., for which the superconducting point would be depressed to  $0^\circ \text{K.}$ , i.e., a frequency for which superconductivity would not exist.

In the above work previously reported the resistance of the specimen was found by observing the reaction of a resonant circuit of the material (in the low-temperature compartment) on the plate current of the primary oscillating circuit which itself provided the original high-frequency current. The resistance was then calculated assuming that the change in the plate current of the primary oscillator at resonance, due to the presence of the resonant circuit, was inversely proportional to the resistance of the specimen. This method required that the specimen coil be coupled quite tightly to the oscillator; it was considered that methods involving looser coupling would give more dependable results.

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### Methods of Measuring High-frequency Resistance

For this reason and also because the plate current depends so largely on the frequency, it was considered desirable to change the experimental technique. Consequently three variations of ordinary methods were used to determine the high-frequency resistance.

1 (a). The schematic diagram of this method is shown in Fig. 1a; this is particularly satisfactory when used with samples which have a low resistance, e.g., solid tin wires. The resonant circuit was made up of the specimen under test and a vacuum-tube voltmeter was used to measure the voltage across a condenser in this resonance circuit. The vacuum tube used in the voltmeter circuit was of a miniature type so that it could be attached closely to the resonance circuit and lowered into the flask where it could be submerged in the liquid helium.

Changes in the plate current in the voltmeter tube were amplified if necessary by the use of another tube outside the helium flask. In this method the output of the oscillator was kept constant and the current through the specimen read from the vacuum-tube voltmeter.

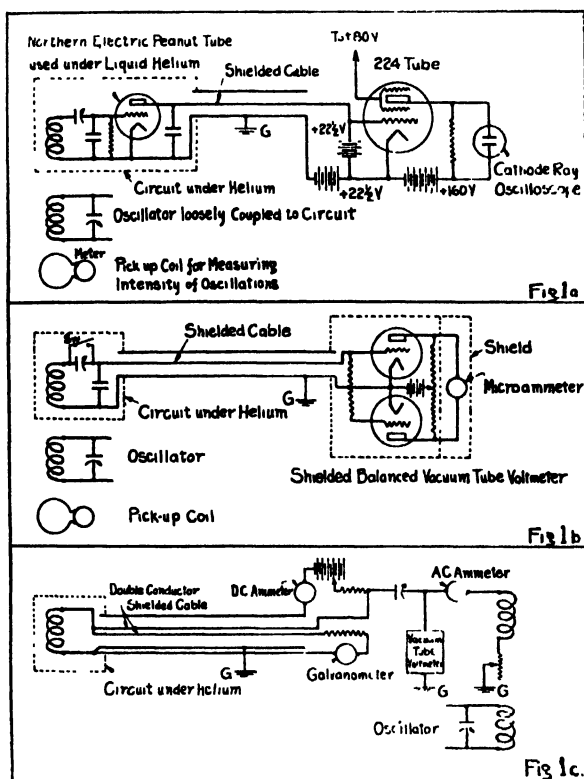


FIG. 1. Methods of measuring high-frequency resistance.

1 (b). In Fig. 1b a balanced shielded detector circuit is shown. Using this method the output of the oscillator was varied to keep the current through the specimen constant. This method was used with very low current strengths.

1 (c). A third method, illustrated in Fig. 1c, while not as sensitive as the two preceding methods, was better for determining the effect of current strength on the resistance. It consisted in sending a measured alternating current through a concentric shielded cable to the coil under test. The voltage across a circuit, consisting of a coil and a condenser of the correct capacity for resonance, is then directly proportional to the sum of the resistances of the coil and the leads. Since the vacuum-tube voltmeter is a "square-law" instrument,

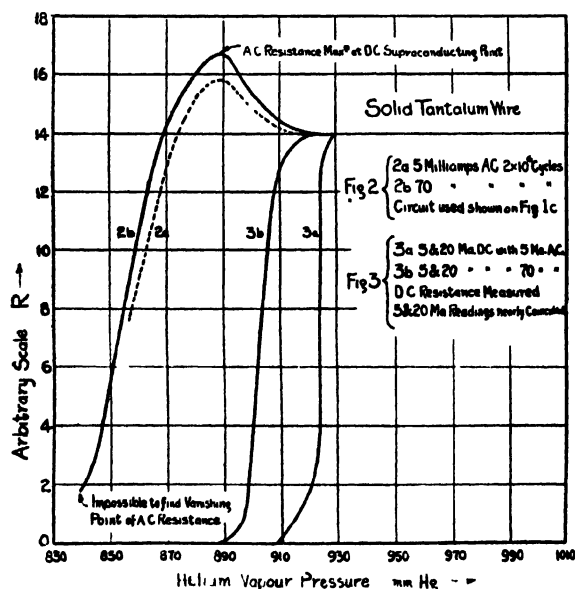
the sensitivity is increased by the presence of additional resistance in the leads, provided this is not too large. In practice this is always less than the resistance of the coil just above the superconducting point. This method is the most satisfactory for measuring the effect of high-frequency currents on the d-c. resistance and the effect of direct currents on the high-frequency resistance. In measuring the latter effect it is essential that the external resistance of the circuit be varied as little as possible since any variation in this resistance will cause a variation in the apparent alternating current resistance of the sample. In the experiments, voltages up to 720 volts d-c. were used to give a maximum current of 24 milliamps. The coil used was non-inductively wound and so designed that its resistance (just above the superconducting point) was not increased by more than 10% by the d-c. circuit.

### Experimental Results

The results reported below may be conveniently divided into the following groups:—

I. *Experiments on solid wires.* (1) Direct currents only; (2) high-frequency currents only; (3) with the superposition of direct and high-frequency currents—(a) measurement of high-frequency resistance, (b) measurement of d-c. resistance.

II. *Experiments on a thin film of tin coated on a fine constantan wire.* (1) Direct currents only; (2) high-frequency currents only; (3) with the superposition of direct and high-frequency currents—(a) measurement of high-frequency resistance, (b) measurement of d-c. resistance.



FIGS. 2 and 3. (2) Curves for high-frequency resistance and (3) for direct-current resistance when high-frequency currents are superimposed.

I (1). In the experiments with solid wires tantalum was used; the measurement of the d-c. resistance was in every case carried out as before by the standard potentiometer method. The d-c. results were the same as those formerly obtained, the drop in the resistance at the transition point being very abrupt.

I (2). In Fig. 2 are shown curves for the change in the high-frequency resistance for currents of 5 and 70 milliamps. respectively; the d-c. resistance curve, which is practically the same as 3a, may be used for comparison. The curve for 70 milliamps. is more dependable than the one for five, but the

curves are almost coincident and show little dependence on current strength for currents of this order.

It is remarkable that the initial change in the resistance at the transition point is a slight increase; this is similar to previous results (2), an effect formerly thought to be due to experimental error. There can be no doubt that this is a real effect for solid wires, but it is not present with thin films of the same materials; this point will be referred to later.

The curves indicate that the reduction in resistance does not appear to set in until a temperature is reached distinctly below that for the d-c. transition.

I (3, a). Small direct currents of the same order of magnitude as the high-frequency currents have no noticeable effect on the transition point for high-frequency resistance.

I (3, b). In Fig. 3 the curves are coincident for direct currents of 5 milliamps. and 20 milliamps.; curve 3a shows the result of superimposing a high-frequency current of 5 milliamps. on these direct currents, while curve 3b is the result of superimposing 70 milliamps. high frequency on the direct currents. The superimposed current had a frequency of  $2 \times 10^6$  cycles per sec. in each case.

From these results it can be concluded that the effect of high-frequency currents on the d-c. resistance measurement varies as the strength of the high-frequency current and, not as formerly thought, directly as the ratio of the direct and the high-frequency current strengths.

In all the papers referred to above considerable attention has been given to the "skin effect" present when dealing with alternating currents, an effect which becomes more and more important as the frequency increases. It was thought possible that this complication could be very much reduced by experimenting on a thin film of superconducting substance—an arrangement which was realized by coating a fine constantan wire with pure tin. This was done by drawing the wire through molten tin and wiping the coating down to as uniform thickness as possible. The remainder of the experiments reported here were performed on these coated wires; as the constantan does not become superconducting and maintains a rather high resistance at even very low temperatures, the coating of tin is equivalent, as far as superconductivity is concerned, to a very thin cylinder of tin.

II (1). For small currents the measurement of d-c. resistance gave the same transition points as measurements of high-frequency resistance gave for frequencies of  $2 \times 10^6$ ,  $10^7$ , and  $3 \times 10^7$  cycles per sec., *i.e.*, wave-lengths of 150, 30 and 10 metres.

For different samples of tin coating on constantan the d-c. transition point was always the same (for small values of the current), but the slope of the curve down to the point of disappearance of the resistance varied slightly with different samples.

A series of experiments was carried out to determine the effect of varying the value of the direct current on the transition point. The results are shown in Fig. 4 which shows curves for four values of the direct current—50, 100, 200, and 500 milliamps. The spread in temperature of the starting points

from Curve 1 to Curve 4 corresponds to about  $0.5^\circ$  K. These results agree with those of Sizoo and Onnes (4) on the properties of thin films of tin sputtered on glass.

II (2). In Fig. 5 are shown curves comparing the measurement of d-c. resistance (Curve 1, current 50 milliamps.) with the measurement, for the same sample, of the high-frequency resistance at two different frequencies; Curve 2,  $10^7$  cycles per sec., 25 milliamps. and Curve 3,  $3 \times 10^7$  cycles per sec., and a current of varying value, but always small. These measurements were carried out by using the second method which gives more dependable results than the original method.

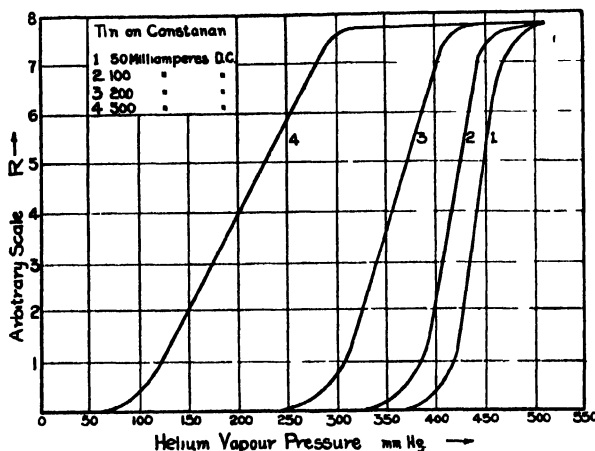


FIG. 4. Variation of superconducting point with current strength.

striking characteristics:—

(1) The transition point is the same for direct and high-frequency currents for small values of the current.

(2) The disappearance of the resistance, or the vanishing point, is at a lower temperature for high-frequency resistance than for d-c. resistance; in fact, the value of the resistance with the high-frequency currents never became absolutely zero, as it seems the d-c. resistance does. This is probably due to induced resistance from the surrounding materials.

(3) There is no indication of a slight rise in resistance before the sample becomes superconducting to high-frequency currents, such as was invariably observed with solid wires (see Fig. 2, a and b).

II (3, a). Using small currents there is no appreciable effect on the transition point for high-frequency resistance due to the superposition of a direct current with the tin-coated wire.

II (3, b). The effect of superimposing high-frequency currents on direct currents while measuring d-c. resistance is to lower the transition or critical point for the sample. To show this effect the temperature was lowered until the d-c. resistance disappeared completely. High-frequency current was

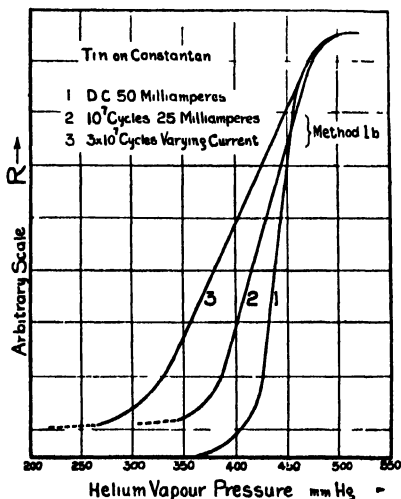


FIG. 5. Resistance of tin on constantan for direct-current and high-frequency currents.

then sent through the sample, the frequency was kept constant, and the value of the high-frequency current necessary to bring the d-c. resistance back to its half-value was determined. Currents of two frequencies, *viz.*, 60 cycles per sec. and  $3 \times 10^6$  cycles per sec., were successively used on the same sample through which was being passed the same direct current, with the following results:— $3 \times 10^6$  cycles per sec. (wave-length 100 m.)—140 milliamps.; 60 cycles per sec. (wave-length  $5 \times 10^4$  m.)—230 milliamps. That is, the values of the alternating currents have the ratio of 2 to 3, while the frequencies change in the ratio of 50,000 to 1.

It is well known that the current distribution in a conductor depends on the frequency of the applied e.m.f. At high frequencies the current density is a maximum at the surface of the conductor and decreases rapidly on approaching the centre. There is a phase variation in the current from the centre of the conductor to the surface; indeed, there may be a difference of  $180^\circ$  or more between the phase at the surface and at the centre of the conductor. In this case a decrease in resistance of a section of material in the centre of the conductor will cause an increase in the resistance of the wire as a whole. This is the same effect as is observed with two tightly coupled circuits connected in shunt in one of which the ratio of resistance to inductive impedance is much less than in the other. From this it is seen that if the centre of a wire becomes superconducting while the resistance of a thin layer on the surface of the wire remains the same, then there may be observed an apparent rise in resistance of the wire as a whole. This then may account for the initial rise of high-frequency resistance observed at the d-c. superconducting point (see Fig. 2a, b). The measurements with solid tin wire are not sufficiently accurate to bring out this effect. Since the current distribution is determined for high frequencies by the effective impedance, not by the resistance of a section of the wire, it follows that there will be a different high-frequency and d-c. superconducting point if there is any variation in this point for different parts of the wire. The d-c. observations will give the superconducting point of whichever part of the wire loses its resistance first. The high-frequency point will be determined by a thin skin on the outside of the wire, the thickness of this film decreasing with increasing frequency.

From this it follows that if there is anything affecting the surface and thereby causing a lowering of its superconducting point, then the apparent superconducting point of a wire would be lower for high frequency than for d.c. There does not seem to be any simple way to test this hypothesis but it does suggest that the only suitable type of conductor is one in which the current distribution is as far as possible *independent* of frequency. The only conductor satisfying this condition is a thin film of the material. It follows that the results obtained with coated wires are more significant than those obtained with solid wires.

From these results it seems certain that there is no appreciable change in the transition point for frequencies up to  $3 \times 10^7$  cycles per sec. The apparent



change is indirectly due to the change in current distribution in the wire and probably directly due to surface changes of the conductor. Further, the effects of a.c. on the d-c. resistance are probably due to magnetic effects and certainly depend only on the magnitude of the alternating current, not on the ratio of alternating to direct currents.

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# THE BAROMETRIC FORMULA FOR REAL GASES AND ITS APPLICATION NEAR THE CRITICAL POINT<sup>1</sup>

By R. RUEDY<sup>2</sup>

## Abstract

According to the theory of the continuity of liquid and gaseous states, as expressed for instance in van der Waals' equation, pronounced density differences may exist in a short column of fluid maintained, throughout its length, at the critical temperature. The point in the tube at which the density of the contents has decreased a given percentage from the critical value is the higher the larger the ratio of the critical temperature to molecular weight. For substances like neon the variations are so large that a measurable separation of isotopes may be expected at or near the critical point; for other substances the computed results are at least of the magnitude found by experiment. Also, according to the theory, in order to obtain, at or near the critical point, a column of gas of uniform density a temperature gradient must be allowed to exist along the column.

## Introduction

The barometric formula for the variation of pressure with altitude as deduced from the laws for perfect gases is in current use and is accurate within the narrow range of pressures  $p$ , volumes  $v$ , and temperatures  $T$  over which it is needed. At higher pressures, however, the deviations from the law for ideal gases necessitate corrections being made. The differences become of particular interest in the neighborhood of the critical point ( $p_c, v_c, T_c$ ), where a slight increase in pressure, such as is caused by the mere weight of thin layers of gas, may cause important variations in the density.

## Gases Conforming to van der Waals' Equation

When the gas or vapor being studied conforms to van der Waals' equation, at least over a limited range of pressures, volumes or densities and temperatures, we have for  $n$  gram-molecules of gas the relation:

$$\left(p + \frac{n^2 a}{v^2}\right) (v - nb) = nRT,$$

or, when the density  $\rho$  is introduced, with  $M$  the molecular weight,

$$pM^3 = \frac{\rho M^3 RT}{M - b\rho} - a\rho^2.$$

On differentiating  $p$  with respect to  $\rho$ , taking into account the relations

$$\begin{aligned} b &= M/3\rho \\ a/b &= 27RT_c/8 \end{aligned}$$

and the fact that an increase of  $dh$  cm. in height means a change in pressure (in atmospheres) equal to

$$dp = -\frac{\rho}{1033} dh,$$

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we obtain

$$\frac{3\rho_0(\rho - \rho_0)}{(3\rho_0 - \rho)(3\rho_0 - \rho_0)} - \left( \ln \frac{3\rho_0 - \rho}{3\rho_0 - \rho_0} + \ln \frac{\rho_0}{\rho} \right) - \frac{9T_0(\rho - \rho_0)}{4T\rho_0} = -\frac{M(h - h_0)}{RT \cdot 1033}$$

instead of the barometric formula

$$\ln \frac{\rho_0}{\rho} = \frac{h - h_0}{1033} \frac{M}{RT}$$

Supposing now that the column of gas is at (or very near) the critical temperature  $T_0$ , and that the density  $\rho_0$  at height  $h_0$  is equal to  $\rho_c$ , the relation becomes

$$\frac{3}{2} \frac{\rho - \rho_0}{2\rho_0 - (\rho - \rho_0)} - \ln \left( 1 - \frac{(\rho - \rho_0)}{2\rho_0} \right) - \ln \frac{\rho_0}{\rho} - \frac{9}{4} \frac{\rho - \rho_0}{\rho_0} = \frac{h - h_0}{1033} \frac{M}{RT_0}$$

When columns of some 20 or 30 cm. are considered the difference in densities  $\Delta\rho_c = (\rho - \rho_0)$  between any two layers is small, and

$$\ln \left( 1 + \frac{\Delta\rho}{\rho} \right) = \frac{\Delta\rho}{\rho} - \frac{1}{2} \left( \frac{\Delta\rho}{\rho} \right)^2 + \frac{1}{3} \left( \frac{\Delta\rho}{\rho} \right)^3,$$

so that leaving out the subscript letter  $c$  in  $\rho_0$ , we obtain an equation which depends only on the ratio  $\Delta\rho/\rho = Y$ , namely,

$$\frac{3}{2} \frac{\Delta\rho}{2\rho - \Delta\rho} - \frac{3}{4} \frac{\Delta\rho}{\rho} - \frac{3}{8} \left( \frac{\Delta\rho}{\rho} \right)^2 + \frac{3}{8} \left( \frac{\Delta\rho}{\rho} \right)^3 = -\frac{M}{RT_0} \cdot \frac{h - h_0}{1033},$$

$$\frac{2Y}{2 - Y} - Y - \frac{1}{2} Y^2 + \frac{1}{2} Y^3 = -\frac{4}{3} \frac{M}{RT_0} \cdot \frac{h - h_0}{1033}$$

or simply

$$\frac{3Y^3 - Y^4}{2 - Y} = -\frac{M}{T_0} \cdot \frac{h - h_0}{0.375 \times 86.1 \times 1033},$$

for a gas conforming to van der Waals' equation, instead of

$$Y - \frac{1}{2} Y^2 + \frac{1}{3} Y^3 - \frac{1}{4} Y^4 = -\frac{M}{T_0} \frac{h - h_0}{86.1 \times 1033}$$

for a perfect gas.

The results obtained for various values of  $Y$  are shown in Table I.

TABLE I

DENSITY VARIATIONS IN A GAS OF MOLECULAR WEIGHT  $M$  NEAR THE CRITICAL TEMPERATURE  $T_0$

Percentage change $Y$	$\frac{M}{T_0} \Delta h$ (cm.)	Percentage change $Y$	$-\frac{M}{T_0} \Delta h$ (cm.)
- 0.5	0.006	0.5	0.006
- 1	0.05	1	0.05
- 2	0.40	2	0.40
- 3	1.34	3	1.36
- 4	3.18	4	3.22
- 5	6.20	5	6.31
- 6	10.71	6	10.92
- 7	16.97	7	17.36
- 8	25.29	8	25.97
- 9	35.95	9	36.71
- 10	49.24	10	50.91

Since few gases conform very closely to van der Waals' equation, these values are to be considered as giving the order of magnitude rather than actual values applying to all substances. But despite this restriction it may be said that for values of  $M/T_0$  not less than 0.1, excepting a few substances such as water (0.028) and hydrogen (0.06), the density may increase or

decrease by several per cent within a few centimetres above or below a layer that is exactly at the critical density ( $M/T_c$  equals 0.146 for carbon dioxide, 0.183 for chlorine, 0.276 for carbon tetrachloride, 0.115 for methyl ether, 0.158 for ethyl ether, 0.45 for neon and for xenon and 0.74 for helium).

The density variations are most rapid directly above and below the layer which is at the critical density, and at, or near, the critical temperature. The effect may, under favorable conditions, simulate a real surface of separation between the lower and the upper part of the column. Helium, for instance, shows a very sharp boundary surface at the critical point in agreement with what may be expected from the large value of  $M/T_c$ .

The point in the tube at which the density of the contents has decreased, or increased, a given percentage below or above the critical value  $\rho_c$  is the more distant from the layer which is at the critical point, the smaller  $M/T_c$ , so that when a gas consists of two isotopes and the critical temperature is nearly the same for both constituents, the heavier molecules must show a tendency to concentrate, in a certain measure, near the layer which is at the critical point.

### Gases Conforming to Wohl's Equation

Although van der Waals' equation continues to be the best simple mathematical expression of the continuity of state, the equation is unsatisfactory when used over a wide range owing to our lack of knowledge of the exact values of  $a$  and  $b$  at different temperatures and pressures. It has been found that for certain substances, carbon dioxide and fluorobenzene, for instance, empirical formulas, which have no theoretical foundation apply over a much wider range than van der Waals' equation. One of them is Wohl's equation (3):

$$\left(p + \frac{a}{v(v-b)} - \frac{c}{v^3}\right)(v-b) = RT,$$

or, using densities  $\rho$  instead of volumes

$$p = \frac{\rho RT}{M - b\rho} - \frac{a\rho^2}{M(M - b\rho)} + \frac{c\rho^3}{M^3},$$

where  $p$  is measured in atmospheres; moreover,  $b = M/4\rho_c$ ;  $a = 8MRT_c/5\rho_c$ ;  $a/b = 32RT_c/5$ ;  $c = 16M^2RT_c/15\rho_c^2$ . Proceeding as before, the barometric formula becomes

$$\left(\frac{RT}{M} - \frac{32RT_c}{5M}\right) \left(\frac{4\rho_c(\rho - \rho_c)}{(4\rho_c - \rho)(4\rho_c - \rho)} - \ln \frac{4\rho_c - \rho}{4\rho_c - \rho_c}\right) + \frac{RT}{M} \ln \frac{\rho}{\rho_c} + \frac{8RT_c}{5M} \frac{\rho^2 - \rho_c^2}{\rho_c^2} = -\frac{h - h_c}{1033}.$$

In the special case where the column of fluid is maintained at the critical temperature, the relation, leaving out the subscript letter  $c$  of  $\rho_c$ , becomes

$$\frac{27}{5} \left( \ln \left( 1 - \frac{\Delta\rho}{3\rho} \right) - \frac{4}{3} \frac{\Delta\rho/\rho}{3 - \Delta\rho/\rho} \right) + \ln \left( 1 + \frac{\Delta\rho}{\rho} \right) + \frac{8}{5} \left( 1 + \frac{\Delta\rho}{\rho} \right)^2 - \frac{8}{5} = -\frac{M}{RT_c} \frac{\Delta h}{1033},$$

where  $\Delta h$  is the difference in level between the layer where the density is  $\rho$  and the layer with the critical density  $\rho_c$ . Apart from the ratio  $M/T_c$ , which

differs from one substance to another, the level  $\Delta h$ , as in the case where van der Waals' equation applies, depends merely on the value of  $Y = \Delta \rho_0 / \rho_0$  whatever the gas concerned, and we may write

$$\ln(1+Y) - \frac{27}{5} \left( \frac{4}{3} \frac{Y}{3-Y} - \ln \left( 1 - \frac{Y}{3} \right) \right) + \frac{8}{5} (1+Y)^2 - \frac{8}{5} = -\frac{M}{RT_0} \Delta h$$

or for small values of  $Y$ :

$$\frac{-5Y^4 + 4Y^3}{3-Y} = -\frac{75}{16} \frac{M}{RT_0} \frac{\Delta h}{1033}$$

for a gas conforming to Wohl's equation, in place of

$$\frac{6Y - 5Y^2}{3-Y} = -\frac{2M}{RT_0} \frac{\Delta h}{1033}$$

for a perfect gas.

It is readily seen that the equation gives 10 to 100 times greater variation of density with height than is predicted by van der Waals' equation. The real gases seem to fall within these two limiting cases (1, 2), although Wohl's equation does not apply at the critical temperature to volumes smaller than the critical value.

### Conclusion

The existence of layers with slightly different temperatures also explains why, in a closed tube containing the weight of liquid just sufficient to fill the available space at the critical point, the boundary surface does not necessarily appear halfway up the tube and why even when the correct amount of liquid is not put in the tube before sealing the sudden disappearance of the meniscus may still be observed above or below the middle of the tube. On the other hand, as a density gradient will also exist, according to the general equations, at temperatures slightly above the critical value, it follows that experiments in closed tubes are not likely to indicate the critical temperature with a high degree of precision. Since such experiments are commonly included, however, as laboratory exercises, a discussion of their meaning with regard to the continuity of liquid and gaseous states would gain from a brief study of the actual effects observed.

Variations of density in a column of gas near the critical point cannot be taken as necessarily disproving the theory of the continuity of state.

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